EPIDEMIOLOGY AND PULMONARY SEQUELAE IN INFANTS WITH SEVERE RESPIRATORY SYNCYTIAL VIRUS LOWER RESPIRATORY TRACT INFECTION

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Declaration

I, Charl Verwey, declare that this thesis is my own, original work. It is being submitted for the Degree of Doctor of Philosophy in Clinical Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

_____

Charl Verwey

_____day of_____20_____in_____

To the sun in my sky,

Heleen Verwey,

and the glittering stars at night,

Esca, Akhil, and Kiran.

Presentations arising from this research report

1. South African Thoracic Society and Pan-African Thoracic Society Combined Annual Congress, 2018, Durban, South Africa
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Abstract

Respiratory Syncytial Virus (RSV) is the commonest cause of lower respiratory tract infection (LRTI) and hospitalization for LRTI in children. RSV LRTI during early childhood may increase susceptibility to recurrent wheezing and asthma. The objective of this study was to determine the pulmonary sequelae at one and two years of age following RSV LRTI hospitalization in term infants.

A longitudinal case-control study was undertaken in Johannesburg from April 2016 to December 2019. Cases constituted infants previously hospitalized with PCR-confirmed RSV LRTI; and controls were well infants not previously hospitalized with LRTI. A questionnaire detailing environmental and medical history, as well as a modified ISAAC questionnaire, was administered, and pulmonary function testing, including forced oscillation technique, tidal breath flow-volume loops, and multiple breath wash-out, was performed, at one and two years of age.

One (n=308) and two-year-old (n=214) cases were more likely than one (n=292) and two-year-old (n=209) controls to have experienced clinical pulmonary symptoms, including wheezing or whistling in the chest, received treatment for wheezing or whistling in the chest, and had any admissions for wheezing or whistling in the chest or any chest infection, after the initial RSV LRTI during infancy.

Pulmonary function testing reported that RSV LRTI during infancy led to an increase in airway resistance by two years, along with a decrease in compliance at both one and two years. There was an increased work of breathing at one year, but this was no longer present at two years. The expiratory time was decreased, while the expiratory flow parameters, as well as the time to peak expiratory flow to total expiratory time ratio were increased. FRC and LCI were abnormal at one year but had returned to normal at two years.

This study described the first set of pulmonary function indices in healthy one and two-year-old black African children from a LMIC.

Children hospitalized with RSV LRTI during infancy had more clinical and pulmonary function sequelae through to two years of age when compared to healthy controls. Whether prevention of RSV LRTI during early infancy would reduce the risk for subsequent pulmonary sequelae warrants further investigation.

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Table of Contents

Nomenclature / List of abbreviations

AFV – Area of flow-volume loop

aOR – Adjusted odds ratio

ARI – Acute respiratory tract infection

ATS – American Thoracic Society

AX – Area under the reactance curve

BD – Bronchodilator

BDR – Bronchodilator response

BPD – Bronchopulmonary dysplasia

BTPS – Body Temperature and Pressure Saturated

CAP – Community acquired pneumonia

CCD – Central conserved domain

C_{et} – End tidal gas concentration

CEV – Cumulative expiratory volume

CFR – Case fatality ratio

CHBAH – Chris Hani Baragwanath Academic Hospital

CI – Confidence interval

CLWH – Children living with HIV

CO_2 – Carbon dioxide

CRF – Case Report form

CrI – Credible interval

Ct – Cycle threshold

DSR – Dead space reducer

EEL – End-expiratory level

EMG – Electromyography

EPI – Expanded programme of immunisation

ERS – European Respiratory Society

ETS – Environmental tobacco smoke exposure

F protein – Fusion protein

FEF_{25-75} – Forced expiratory flow between 25–75% of FVC

FEF_{75} – Forced expiratory flow at 75% of FVC

FEV_1 – Forced expiratory volume in one second

FOT – Forced oscillation technique

$FRC_{(He)}$ – Functional residual capacity measured though Helium gas dilution technique

$FRC_{(Pleth)}$ – Functional residual capacity measured through body plethysmography

F_{RES} – Resonance frequency measured through FOT

FVC – Forced vital capacity

G protein – Attachment protein

GA – Gestational age

GBS – Group B streptococcus

HEU – HIV exposed uninfected

HiB – *Haemophilus influenzae* type B

HIC – High-income country

HIV – Human immunodeficiency virus

HMPV – Human metapneumovirus

HREC – Human Research Ethics Committee

HUU – HIV unexposed uninfected

ICU – Intensive care unit

ID – Identification number

IETS – Intrauterine environmental tobacco smoke exposure

IFN – Interferon

IgE – Immunoglobulin E

IGIV – Immune globulin intravenously

IL – Interleukin

IMCI – Integrated management of childhood illness

IOS – Impulse oscillometry

IQR – Interquartile range

IS – Induced sputum

ISAAC - International Study of Asthma and Allergies in Childhood

L – Length

LCI – Lung clearance index

LCI 2.5 – Lung clearance index at 2.5% of the original gas concentration

LCI 5 - Lung clearance index at 5% of the original gas concentration

LMIC – Low-to-middle income countries

LRTI – Lower respiratory tract infection

M1M0 – First moment analysis of the washout curve

M2M0 – Second moment analysis of the washout curve

MAC – Medical Advisory Committee

MBW – Multiple breath washout

MEF_{25} – Maximal expiratory flow at 25% of FVC

MTEF – Mean tidal expiratory flow

MTIF – Mean tidal inspiratory flow

NHLS – National Health Laboratory Service

NK – Natural killer

NPA – Nasopharyngeal aspirate

NPS – Nasopharyngeal swab

O_2 – Oxygen

P_{acin} – Maximum contribution of (First breath slope of phase III) - (First breath turn-over x Slope of SnIII versus turn-over)

PCP – *Pneumocystis jirovecii*

PCR – Polymerase chain reaction

PERCH – Pneumonia Etiology Research for Child Health

PFT – Pulmonary function test

PICU – Paediatric intensive care unit

PIV – Parainfluenza virus

PRISMA-P – Preferred Reporting Items for Systematic Reviews and Meta-Analyses-Protocol

PTEF – Peak tidal expiratory flow

PTIF – Peak tidal inspiratory flow

R_{aw} – Airway resistance measured through body plethysmography

R_{eE} – End-expiratory resistance

R_{eI} – End-inspiratory resistance

R_{Hz} – Resistance at a particular frequency measured through FOT

R_{Int} – Resistance measured through interrupter technique

R_{mean} – Mean airway resistance

RMPRU – Respiratory and Meningeal Pathogens Research Unit

RNA – Ribonucleic acid

RSV – Respiratory syncytial virus

R_t – Total resistance measured through FOT

RTHC – Road to Health Card

S_{acin} – (First breath slope of phase III) - (First breath turn-over x Slope of SnIII versus turn-over)

SAMRC – South African Medical Research Council

S_{cond} – Slope of SnIII versus turn-over

SD – Standard deviation

SF_6 – Sulphur hexafluoride

SH protein – Small hydrophobic protein

SQL - Structured Query Language

TBFVL – Tidal breath flow volume loops

T_E – Total expiratory time

TEF_{10} – Flow at 10% of V_T remaining

TEF_{25} – Flow at 25% of V_T remaining

TEF_{50} – Flow at 50% of V_T remaining

TEF_{75} – Flow at 75% of V_T remaining

TH – Helper T-lymphocytes

T_I – Total inspiratory time

TIF_{50} – Inspiratory flow at 50% of V_I

TLR – Toll-like receptor

TNF – Tumour necrosis factor

TO – Turnover

TPEF – Time to peak tidal expiratory flow

TPIF – Time to peak tidal inspiratory flow

T_{Tot} – Total breath time

U5MR – Under-5 mortality rate

UR – Uncertainty range

UTM – Universal transport medium

$\dot{V}$ - Flow

V – Volume

$\dot{V}_E$ – Expiratory minute ventilation

V_E – Expiratory volume

V_I – Inspiratory volume

VPEF – Volume at peak tidal expiratory flow

VPIF – Volume at peak tidal inspiratory flow

V_T – Tidal volume

WHO – World Health Organization

WITS – University of the Witwatersrand

X_{eE} – End-expiratory reactance

X_{eI} – End-inspiratory reactance

X_{HZ} – Reactance at a particular frequency measured through FOT

X_{mean} – Mean airway reactance

Z_{rs} – Respiratory system impedance

List of Figures

Chapter 1

Chapter 2

Chapter 3

List of Tables

Chapter 1

Chapter 2

Chapter 3

CHAPTER 1

1.0 Introduction

1.1 Burden and aetiology of lower respiratory tract infections

The global under-5 mortality rate (U5MR) has decreased from 77 per 1000 live births in 2000 to 38 per 1000 live births in 2019 over the past two decades (1). This was largely from an emphasis on the home care and appropriate management of newborns, the introduction of the integrated management of childhood illness (IMCI), the expanded programme of immunisation (EPI), and emphasis on childhood nutrition (1, 2). Nevertheless, there were still 5.2 million deaths in children less than five years of age in 2019, roughly half of these occurred in sub-Saharan Africa (3). The South African U5MR has similarly fallen from 78.1 per 1000 live births at the peak of the HIV epidemic in 2003 to 34.5 per 1000 live births in 2020 (4, 5).

The leading cause of death in children less than five years of age outside the neonatal period (0-28 days) is lower respiratory tract infections (LRTI), accounting for approximately 653 000 (12.1%) deaths globally; children from low-income countries are more likely to demise from LRTIs than those from high-income countries (1, 2). Similarly, in South Africa, LRTI (specifically pneumonia) accounts for 9.9-16.9% of the U5MR (4). In the peak of the HIV era, approximately twenty thousand respiratory deaths were reported annually amongst South African children less than five years of age, 76% occurred in children less than one year of age (6). In a recent prospective observational study from Soweto using minimally invasive tissue sampling to investigate the causes of in-hospital deaths in 127 children between one month and 14 years of age (median age of 11 months), pneumonia was reported as the most common immediate cause of death (33.9%); 73% were community-acquired pneumonia (CAP) (7). Furthermore, 36.4% of deaths were in children born to HIV-infected mothers, and 12.8% of deaths were in HIV-infected children (43.8% diagnosed with pneumonia). A nested case-control study of children from a peri-urban community in Cape Town, South Africa, reported a pneumonia incidence of 0.27 (95% Confidence interval (CI): 0.24-0.31) episodes per child year (8). The highest incidence was in the 1-6 month age group (0.54; 95% CI 0.46-0.62 episodes per child year).

Viruses are the commonest (80%) cause of LRTI in young children (9). Large pneumonia aetiology studies, using a host of diagnostic molecular and culture testing techniques, have

however highlighted the polymicrobial nature of LRTIs (8, 10-13). Attributing causality of LRTI to organisms detected in the upper airway is often challenging. Nevertheless, with the identification of specific pathogens, one can assign them as being attributable in the pathogenesis of LRTI, including respiratory syncytial virus (RSV), parainfluenza virus (PIV), human metapneumovirus (HMPV), influenza virus, *Streptococcus pneumoniae, Haemophilus influenzae* type B (HiB), *Haemophilus influenzae* non-type B, *Bordetella pertussis* and *Pneumocystis jirovecii* (PCP) (8, 10, 13). However, for *Streptococcus pneumoniae and Haemophilus influenzae,* this is not the case when using nasopharyngeal sample collection.

The importance of viral pathogens, and particularly RSV, in the aetiology of CAP was also highlighted in the Pneumonia Etiology Research for Child Health (PERCH) study. The PERCH study was a multicenter case-control study reporting on the aetiology of World Health Organization (WHO)-defined severe and very severe pneumonia in children (1-59 months of age), in seven low-resourced settings, including Soweto, South Africa (10). HIV-uninfected cases (n=3981) and community controls (n=5102) were enrolled from August 2011 to February 2014. Viral pathogens were more commonly attributed as the cause of LRTI (61.4%; 95% credible interval (CrI): 57.3-65.6) than bacterial pathogens (27.3%; 95% CrI: 23.3-31.6) in cases with an abnormal chest x-ray. Compared to the overall aetiology, viruses were proportionately less commonly (54.5%; CrI: 47.4-61.5) implicated in the aetiology of very severe pneumonia, and bacterial pathogens more commonly so (33.7%; CrI: 27.2-40.8).

RSV was the most common cause of LRTI (31.1%; 95% CrI: 28.4-34.2) in all cases regardless of the chest x-ray classification. Similarly, RSV was the most common virus associated with pneumonia (27.8%); followed by influenza, boca, adeno and parainfluenza viruses in a nested case-control study of children from a peri-urban community in Cape Town (8). Importantly, only approximately 14% of pneumonia in these two South African studies were caused by pathogens that are currently covered by licensed vaccinations (HiB, *Streptococcus pneumoniae, Bordetella pertussis, Mycobacterium tuberculosis*, and influenza A + B), highlighting that further investment into the development of vaccines targeting prevalent organisms for LRTI is needed to further reduce the U5MR, especially in low-to-middle-income countries (LMIC).

Similar aetiological findings were reported in high-income countries (HIC); for example, in Australia, viruses accounted for 44.4% (95% CI: 33.8-53.3) of childhood (0-18 years) CAP

requiring hospitalization; RSV accounted for almost half of these (20.2%; 95% CI: 14.6-25.5%) (14).

1.2 Epidemiology of RSV infection

Although RSV is the commonest aetiological agent for acute LRTI, its epidemiological impact extends beyond the acute illness and healthcare utilization. There is emerging evidence of the respiratory sequelae and economic burden associated with LRTI in infancy.

The global burden of RSV infection in children less than five years of age has been well described in a few systematic reviews of the past two decades. In a systematic review and meta-analysis published in 2005 (15); an estimated 33.8 million (95% CI: 19.3-46.2) new cases of non-severe RSV-associated infections and 3.4 million of severe RSV-associated LRTI were reported annually, >90% of these episodes occurring in developing countries. RSV-associated LRTI was estimated to cause between 66 000 and 199 000 deaths. An expansion of the above review in 2017 (16); included 326 published and unpublished studies reporting on community incidence, hospital admissions, and in-hospital case fatality ratios (CFR) for confirmed acute RSV LRTI. It was estimated that globally, in 2015, 33.1 million (uncertainty range (UR): 21.6-50.3) RSV-associated LRTI occurred in children under five years of age, of which 30.0 million (95% CI: 19.1-47.0) occurred in LMIC and 2.8 million (95% CI 1.3-6.1) occurred in HIC. There were 3.2 million (UR: 2.7-3.8) hospital admissions with 1.0 million (UR: 0.6-1.6) severe RSV LRTI cases with hypoxaemia, of which 58% occurred in children younger than six months of age. The in-hospital RSV mortality was estimated at 59 600 (UR 48 000-74 500), half occurred in infants younger than six months of age. These reviews highlight the discrepant burden of severe RSV disease between LMIC and HIC.

In a separate systematic review and meta-analysis of studies between 2002 and 2014 that described global incidence rates from 24 countries (17), RSV-associated acute respiratory tract infections (ARI) hospitalizations was reported as 20.02 per 1000 children per year (95% CrI: 9.65-41.31) among children younger than six months of age, 19.19 (95% CrI: 15.04-24.48) among children less than one year of age, and 4.37 (95% CrI: 2.98-6.42) among children less than five years of age. The global CFR was 6.60 (95% CrI: 1.85-16.93) per 1000 among children

less than one year of age and 6.21 (95% CrI: 2.64-13.73) per 1000 among children less than five years of age.

The prevalence of RSV ARI varied widely between and within countries (0.4% to 60.4%) in a systematic review and meta-analysis specifically for African countries (18). The overall RSV prevalence was 14.6% (95% CI 13.0-16.4) in a pooled sample of 154 000 participants with LRTI from around the continent.

In South Africa, the RSV incidence in infants less than six months of age was reported as 0.15 (95% CI: 0.11-0.20) episodes per child year (19). Infant mortality from RSV LRTI was 143.4 (95% CI 0-194.8) per 100 000 population, of which half occurred outside of hospital (20). Furthermore, 6.0% of all annual respiratory deaths in children less than five years of age was due to RSV, of which a quarter occurred outside of hospital (21). This indicates that even though the reported incidence and CFR of RSV-associated LRTI in LMIC is much higher than those in HIC, there may still be underreporting of cases and deaths due to a substantial percentage of the cases and deaths occurring outside of healthcare systems. This was supported by a study that used minimally invasive tissue sampling to investigate the causes of in-hospital deaths in 127 children between one month and 14 years of age. RSV was the most commonly identified pathogen in CAP deaths (21.9%) and RSV-associated deaths occurred exclusively in the first year of life (7).

Furthermore, children living with HIV (CLWH) were 1.4 and 3.7-fold more likely to be hospitalized with LRTI then HIV exposed uninfected (HEU) and HIV unexposed (HUU) children, as well as more likely to be admitted for longer and to die in-hospital. The estimated RSV-associated mortality rate for all-cause deaths in children less than five years of age was also higher in those with HIV (22).

1.3. Economic cost associated with RSV infection

Apart from the associated morbidity and mortality associated with RSV, investigators have also attempted to delineate the substantial economic costs associated with RSV infection and LRTI. In a retrospective case-control study in the USA between August 2013 and August 2014, irrespective of age, cases with an RSV event (defined as hospital stays, emergency room/urgent care visits, ambulatory visits and outpatient visit) had a higher healthcare resource use and annual

financial cost than controls (p<0.0001) (23). In another US study, the mean reported costs of RSV-associated hospitalizations was $40 000 for preterm infants <29 weeks gestation, and $9 000 for full-term infants (24). There have been no studies from LMIC describing the financial burden of RSV-associated infection or hospitalization.

1.4 Historical perspective, taxonomy and microbiology of RSV

Human RSV, then called Chimpanzee Coryza Agent, was first described after being isolated from the upper respiratory tract of a chimpanzee in 1955 (25). Subsequently, in 1956, it was isolated from humans and identified as a virus associated with bronchiolitis in children (26). RSV has recently (2016) been reclassified as an Orthopneumovirus, in the Pneumoviridae family, within the Mononegavirales order (27) (Figure 1.1). Two further viruses, bovine respiratory syncytial virus and murine pneumonia virus belong to the genus Orthopneumovirus, and these, together with a further two viruses, avian metapneumovirus and human metapneumovirus of the genus Metapneumovirus, complete the family Pneumoviridae.

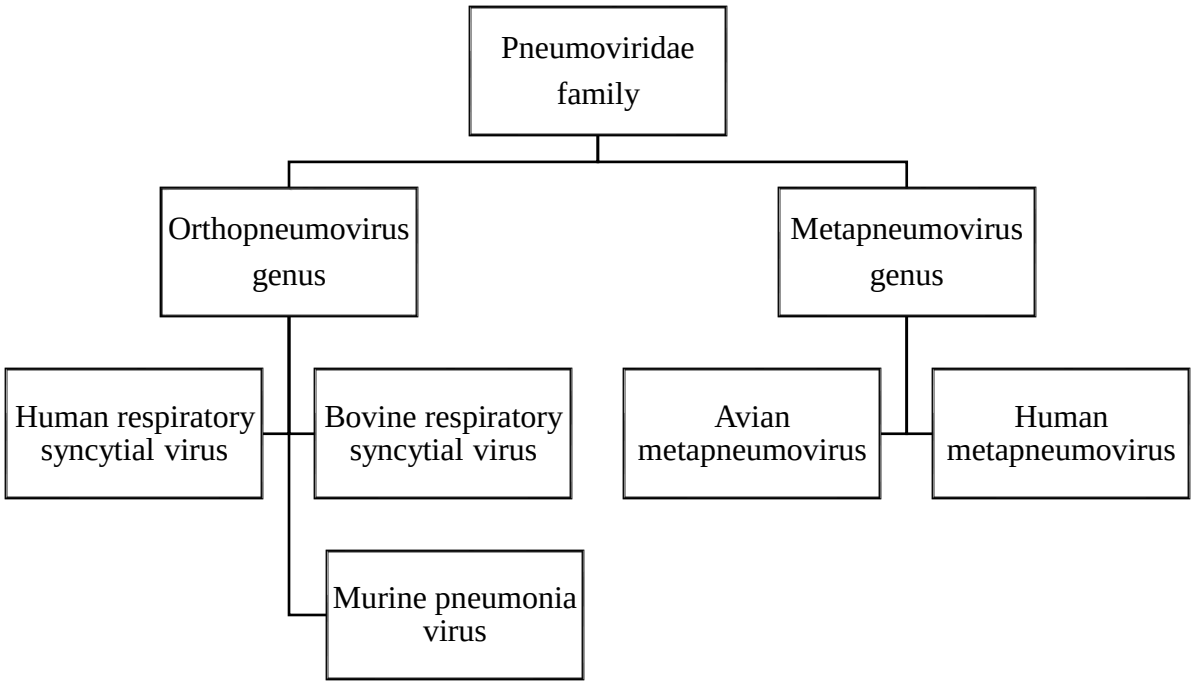


Figure 1.1 Taxonomy of respiratory syncytial virus

RSV is a single-stranded, negative-sense ribonucleic acid (RNA) virus, which is enveloped by a host plasma membrane-derived lipid bilayer. The 15.2 kilo-base pair non-segmented and tightly encapsidated genome contains 10 genes that encode for 11 proteins, three of these are transmembrane glycoproteins: the fusion protein (F protein), the attachment protein (G protein), and the small hydrophobic (SH) protein (28, 29). The F protein is a type I integral membrane glycoprotein that mediates viral penetration into the cell, mitigates fusion between viral and cell membranes, and infected neighboring cells (30). The F protein has two unique conformations, a stable pre-fusion structure, and after binding to its host cell, a highly stable post-fusion structure (30, 31). The F protein has six main antigenic epitopes on its surface (Ø and I-V). Antigenic epitope sites Ø, III and V are only exposed during the pre-fusion F protein conformation, while I, II and IV are exposed on both the pre- and post-fusion F protein conformations (30, 31). The F protein plays a role in activating human leukocytes and thereby initiating the innate immune response to RSV. Palivizumab, a human monoclonal antibody used in the prevention of RSV, and motavizumab, a potent derivative of palivizumab, bind at the antigenic epitope site II.

The G protein was first described as an attachment protein in 1987 (32). It is a carbohydrate rich (60%), heavy glycosylated protein structure that mediates the binding of RSV to the respiratory epithelial cell. It contains a 40 amino acid central conserved domain (CCD) that is not glycosylated and has a central role in the pathogenesis of the RSV infection (33). The G protein is a less efficient neutralization antigen than the F protein (34). It is also found in a secreted form, and these secreted forms act as an antigen decoy to help the virus escape neutralizing antibodies (35, 36).

RSV is classified into two antigenic subtypes, RSV A and RSV B, based on the reactivity of monoclonal antibodies directed at antigenic epitopes against the main transmembrane glycoproteins, especially the F and G proteins (37, 38). Through analyses of the RSV B G protein's genetic variability, it is estimated that these two major subtypes diverged approximately 350 years ago (39). The F protein is highly conserved among RSV isolates from both A and B subtypes, with similar amino acid sequence identities of 90% or higher (30). Numerous genotypes, which can co-circulate during the same RSV season, have been identified within each subtype, with dominant genotypes generally being replaced in successive years (40,

41). There is also a shift in the dominant circulating subtype (A + B) over cycles of one to two seasons (41, 42).

1.5 Pathophysiology and immune responses to RSV infection

RSV can cause a wide range of respiratory tract infections ranging from asymptomatic upper respiratory tract infection to severe LRTI requiring hospitalization (43, 44). The clinical syndrome of bronchiolitis is the most common serious disease manifestation, but the whole spectrum of LRTI, including pneumonia, may occur (43).

RSV infection is transmitted through droplet spread or direct contact with fomites from contaminated surfaces. Inoculation is usually through the nasopharyngeal mucosa or the conjunctival membranes (45). The mean incubation in the nasopharynx is five days, after which the virus spreads via intracellular transmission, cilial motion, or aspiration of nasopharyngeal secretions to the rest of the airways (45-48). In infants, 2-3 days after the onset of upper respiratory tract symptoms, approximately a third will have spread of the infection to the lower airways, especially the apical surfaces of ciliated columnar epithelial cells found in the terminal bronchioles (49).

RSV displays a direct cytopathic effect on the host's airway epithelial cells characterized by epithelial destruction and loss of ciliary motion, as well as a multitude of indirect effects mitigated by the host's own immune response (50).

Host airway epithelial cells recognize RSV through Toll-like receptors (TLR) (51). The virus also binds to the CX3CR1 receptor, for which the CX3C motif on the central region of the G protein has a high affinity (52). The activated receptors lead to the secretion of inflammatory cytokines, which in turn leads to the activation of the initial innate immune response. Interleukin-8 (IL-8) secretion attracts neutrophils to the area of RSV infection (53, 54), while CXRCR1 binding leads to leukocyte chemotaxis. Neutrophils are the dominant cells in the airways of the RSV infected individual (54). Macrophages are drawn into the area leading to the secretion of IL-1B, which in turn leads to the influx of natural killer cells (NK cells) to the area (55). NK cells produce interferons (IFN) that are cytotoxic to viral cells. Tumour necrosis factor (TNF), as well as IL-6 levels are also increased during the initial phase of the RSV infection (56). Through the action of

cytokines and chemokines secreted by the above-mentioned cells, CD4+ helper T-lymphocytes (TH) and CD8+ cytotoxic T-lymphocytes are drawn to the site of infection. This in turn leads to the initiation of the humoral and cellular immune responses and eventually to the CD8+ cells clearing the virus from the host. Animal studies of RSV infection indicate that although CD8+ T-lymphocytes are crucial in the clearing of the virus from the host, an overly robust CD8+ response may contribute to severity of the infection (57), albeit, this being contentious (58-60). The cellular infiltrate contributes to the submucosal oedema experienced and therefore to the characteristic bronchiolar obstruction experienced (61). Bronchoalveolar lavage fluid from an infected individual reveals mostly polymorphonuclear leukocytes, whereas tissue sections reveal lymphocytes as the dominant cells in the peribronchiolar and perivascular spaces (62).

CD4+ T-lymphocyte responses can be either of TH1 or TH2 sub-class. Common TH1 cytokines such as IL-2, TNF alpha, IFN gamma and IL-12 play an important role in the regulation of the adaptive immune response, through self-stimulation and inhibition responses. In RSV infection, a dominant TH1 response is associated with a decreased severity of infection, while a dominant TH2 (cytokines: IL-4, IL-5, and IL-13) response stimulates a more eosinophil based immune response and can lead to more severe RSV disease (63, 64). In addition, asthma and atopy are associated with a dominant TH2 response. There is conflicting data as to whether RSV infection induces a TH1 or TH2 dominant response or whether the balance between these are influenced by other factors including age of infection and genetic susceptibility for atopy (65-68). CD4+ T-regulatory cells however play an important role in host RSV response through suppression of the adaptive immune response with production of the regulatory IL-10 cytokine, which suppresses the function of other cells involved in immune responses (69, 70).

RSV has developed many mechanisms to avoid detection and eradication by the host's immune system. These mechanisms ensure the continued struggle with treating and managing young patients who almost universally get infected with RSV.

1.6 Seasonality and clinical features of RSV infection

RSV infection follows a seasonal pattern, with infection rates in temperate areas peaking in the autumn-winter months, and in tropical areas in the rainy season (71-73). In Johannesburg, South

Africa, the RSV season occurs at the end of the rainy season with cases gradually increasing and maintaining a peak plateau from March (autumn) to May (winter) (73). The RSV season lasts for a median of 4.6 months (95% CI 4.3-4.8) in temperate and tropical climates (74).

Most children are infected with RSV during the first year of life and virtually all by two years of age (75, 76). Reinfection occurs frequently during the first few years of life, and then every 3-10 years through-out life, but these infections tend to diminish in severity (77, 78). Protection against RSV infection is conferred mainly by neutralizing antibodies, with a positive correlation between high titres of serum neutralizing antibodies and protection, and an inverse correlation with risk of infection in children (75, 78).

Symptoms of a RSV upper respiratory tract infection, such as rhinorrhoea, generally occur one to three days before the onset of symptoms of a LRTI, especially a bronchiolitis, such as tachypnoea, wheezing or crepitations. RSV can cause the full spectrum of respiratory tract disease, ranging from an upper respiratory tract infection to acute LRTI, especially bronchiolitis, but also pneumonia, and can be fatal in a small percentage of cases (63).

In a study of 645 Chinese children it was found that coryza, poor appetite, vomiting, diarrhoea, and the typical features of bronchiolitis, dyspnea and wheezing, were more prominent after RSV A infection, while headaches, muscle pain and skin rashes more common after RSV B infection (79).

1.7 Risk factors associated with RSV infection severity

Both the magnitude and intensity of infection, as well as the host response to RSV infection determine the severity of the disease (63). Risk factors for severe RSV disease can be divided into host, environmental and viral factors.

Host related risk factors for severe RSV infection include male gender, age less than six months and malnutrition, whereas breastfeeding has been shown to be protective (72, 80, 81). Host genetic factors may also play a role (82); studies have reported both positive and negative associations between gene polymorphisms and responses to RSV infection (83-86). Certain coexisting medical conditions such as prematurity, congenital cardiac disease with increased

pulmonary blood flow, chronic lung diseases including bronchopulmonary dysplasia (BPD), chromosomal abnormalities such as Trisomy 21, congenital malformations and primary or secondary immunological suppression, including HIV infection (72, 73, 80, 87-89) are risk factors for severe disease. Even though the majority of RSV hospitalization occurs in healthy full term infants (53% at 30/1000 live births per year) during the first year of life, the presence of BPD (388/1000 live births per year), congenital heart disease (92/1000 live births per year) and prematurity (66/1000 live births per year) increases the risk for hospitalization (72, 80). HIV infected children are at increased risk of severe RSV infection, longer duration of hospitalization, and increased case fatality (6, 90-92), and may also experience prolonged shedding of RSV (93, 94). There is a paucity of data on the burden of RSV infection in HEU infants. One study reported that HEU children were more likely to be hospitalized and to have higher odds of death compared to HUU (95).

Demographic and environmental risk factors include; low socio-economic status, household crowding, presence of school age siblings, crèche attendance, and indoor tobacco smoke exposure (72, 80, 81, 96). A prospective longitudinal study from the USA investigating 255 children (median age of nine months) who attended full-time daycare facilities found that 50% of children had RSV detected within six days of the RSV infection in the index case (96).

It has been suggested that infections caused by RSV A are associated with more severe disease than those caused by RSV B (97-102). Furthermore, it has been postulated that the genotype and not just the subtype of RSV may be the determining factor contributing to disease severity (63). A prospective observational study conducted over five respiratory virus seasons found that there was no significant difference in disease severity between RSV A and RSV B subtypes, but that the presence of the RSV A/GA5 genotype was associated with prolonged length of hospital stay (103). It has also been reported that higher RSV viral loads at time of infection are positively correlated with severity of disease (104-106).

The abovementioned risk factors are also an important determinant of the development of pulmonary sequelae after the initial RSV infection episode (107-110).

1.8 Management and prevention of RSV infection

Although multiple treatment modalities have been used in the treatment of RSV infection, there is still no specific curative treatment, and management of RSV LRTI is usually supportive, with oxygen therapy and nutrition (111-117). Management can be divided into measures to prevent acquisition of disease through passive and active immunization; or into active treatment options. Although multiple treatment modalities have been attempted during acute RSV infection, including nebulized hypertonic saline, inhaled, nebulized, or intravenous beta$_2$-agonists, nebulized adrenalin, nebulized ipratropium bromide, montelukast, inhaled, oral, intramuscular or intravenous corticosteroids, and ribavirin (111, 112, 117-125), none have been shown to be effective in treating RSV.

Prevention of RSV through passive immunization can be achieved with the administration of either polyclonal or monoclonal RSV-neutralizing antibodies. RSV-immune globulin intravenously (RSV-IGIV) was the first product used commercially (1996) and consisted of purified polyclonal antibodies sourced from donors with high-titre RSV neutralizing activity. Monthly RSV-IGIV administration in children with high-risk for developing severe RSV disease led to a decrease in the incidence of RSV LRTI, severe RSV LRTI, RSV hospitalization, duration of hospitalization, and duration of RSV-associated ICU admission (126, 127). This was followed by the development of palivizumab, a humanized monoclonal antibody directed at RSV Protein F epitope site II. Palivizumab resulted in a 55% decrease in RSV hospitalization, compared to placebo, when administered monthly for five months to premature children or those with BPD (128). Furthermore, it resulted in decreased RSV admission days, fewer days requiring oxygen, and fewer admissions to the ICU. Palivizumab has been further modified by in-vitro affinity maturation to create motavizumab, a monoclonal antibody that, compared to palivizumab, has 70 times the affinity for the RSV F Protein, resulting in 20 times the in-vitro RSV neutralization (129). Motavizumab resulted in a 26% reduction in RSV hospitalization and a 50% reduction in medically attended acute LRTI in preterm infants (130). Motavizumab was, however, associated with an increase in hypersensitivity reactions in recipients and this, in 2010, led to the United States Food and Drug Administration not granting licensure. A recent study showed that a single dose of Nirsevimab, a recombinant human G1 monoclonal antibody with affinity for the highly conserved site Ø of the prefusion RSV F protein, decreased the incidence of medically attended

RSV-associated LRTI and RSV-associated LRTI hospitalization in preterm infants by 70.1% and 78.4% respectively when compared to placebo (131).

The pathway to RSV vaccines has been delayed because of the 1966 inactivated whole virus RSV vaccine that was associated with more severe disease in infants subsequently infected by the virus (132). In particular, sero-negative children who received the RSV vaccine, were at greater risk of hospitalization (80% vs. 5%; including two fatal cases) than the placebo recipients, after exposure to wild-type RSV virus the following season (62, 133). There are currently multiple RSV vaccine candidates in various stages of production and testing, including maternal vaccination candidates, whose aim is prevention of RSV infection through maternal-fetal antibody transfer, thereby preventing RSV infection during the early months of infancy when the disease is often at its worst (134-136). Most vaccines will aim to stimulate production of neutralizing antibodies targeting either the F or G glycoproteins on the surface of RSV. The different vaccine types currently under investigation include:

1. Nucleic acid vaccines (Messenger RNA vaccines)
2. Live-attenuated / chimeric vaccines usually contain an abnormally cleaved RSV G protein, reducing the infectivity of in vitro human airway epithelial cells dramatically (137, 138).
3. Nanoparticle protein based vaccines. In a randomized controlled trial of a recombinant nanoparticle RSV F protein vaccine, administered to healthy pregnant women between 28 and 36 weeks gestation, Madhi et al. reported that pre-specified criteria for efficacy against the primary endpoint of RSV-associated, medically significant LRTI in infants up to 90 days of life were not met, although a vaccine efficacy of 39.4% was reported (136).
4. Subunit F-protein vaccines.
5. Recombinant vector vaccines.

Large strides in RSV vaccine development have recently taken place, as portrayed through the number of candidate vaccine types now available, as well as the number of studies being conducted. The continuation of these studies and the eventual successful production of an RSV vaccine remains of paramount importance, even more so in LMICs where the burden of disease is increased, the medical resources are decreased and the cost of the cost of passive immunization is prohibitive.

1.9 Pulmonary sequelae of RSV infection

While RSV LRTI mortality is relatively low, with the majority of children recovering uneventfully, the global impact of RSV hospitalization has shifted towards the long-term morbidity associated with RSV LRTI in infancy. Children may be at increased risk of developing recurrent wheezing, altered lung function and chronic respiratory illnesses like asthma. Although it is established that recurrent episodes of wheezing may occur after RSV infection, especially after severe RSV infection, the association between severe RSV infection and confirmed asthma is less well defined.

The determination of the long-term pulmonary sequelae of RSV infection can broadly be divided into two approaches. The first is subjective measurement through administration of a questionnaire to patients or patient caregivers detailing the symptoms or sequelae that the patient has experienced over a certain amount of time after the initial index RSV episode. This approach adds valuable information, such as the presence of wheeze, the physician's diagnosis of asthma, and current treatment for asthma. Administration of a questionnaire however has its shortcomings: (i) patient and parental recall diminishes with time, potentially leading to under-reporting of signs or symptoms, or conversely over-reporting in individuals who may have had previous contact with study or hospital personnel; (ii) definitions applied in questionnaires may vary between individual studies, potentially including or excluding participants based on these differences and often making interpretation of results between studies less reliable; and (iii) none of the questions are based on objective measurements.

The second approach is objective measurement through performance of pulmonary function testing after a specific time period following the index RSV infection. These measurements, although providing strong, robust and standardized data also have limitations. Firstly, expensive equipment with experienced operators are required, making them less appealing in LMICs where the burden of RSV disease is higher. Secondly, the tests are time consuming and generally require some form of patient interaction, such as the use of a patient-facemask interface, which is often problematic in young children. Thirdly, the results obtained between different pulmonary function tests are very rarely comparable, due to the measurement of different physiological parameters, as well as selective reporting practices. Lastly, due to the developing lungs of the

child with rapid maturational changes during the first decade of life, results between different age groups are often difficult to compare, even though the general trend of the results may be similar, and normative reference ranges are not always available for all the age-groups and different pulmonary function techniques.

Nonetheless, administration of a questionnaires and pulmonary function testing are valuable tools for identifying and reporting pulmonary sequelae. In the following subsections, we provide a literature review on the association between RSV LRTI and sequelae (wheezing, asthma and abnormal lung function test)

1.9.1 RSV and wheezing

In an early study that reported on 55 children two years after admission for bronchiolitis (49% RSV confirmed), 75% had reported wheezing and 13% had a subsequent admission for wheezing (139). These findings were mirrored in a retrospective study, where 60% of 292 children younger than three years of age hospitalized with RSV or Influenza A reported multiple episodes of wheezing within one year after hospitalization and 15% required a subsequent admission for wheezing (140). The severity of the initial infection, as judged by the need for intensive care unit (ICU) admission, was the single most significant risk factor for the development of subsequent wheezing.

Further evidence of an independent increased risk of recurrent wheezing was demonstrated in a large retrospective review of ~70 000 term infants in the USA with RSV LRTI during infancy (109, 141). Recurrent wheezing was associated with both RSV infection requiring outpatient management (Adjusted odds ratio (aOR) 1.38; 95% CI: 1.03-1.85) and with RSV infection requiring hospitalization (aOR 2.59; 95% CI: 1.49-4.50) (109). Additionally, a well-designed prospective case-control study from Germany comparing 42 cases with 84 controls reported an increased risk of recurrent wheezing during the first year of life following RSV LRTI hospitalization (15.5% vs 3.6%) (142). A case-control study from the United Kingdom reported results from 180 ten-year-old children admitted for RSV infection during infancy (143); 42% of the cases had further wheezing episodes compared to 19% of controls. At 10 years of age,

however, there was no difference between the two groups in the number of children who were receiving treatment for asthma.

Another longitudinal birth cohort study, described an increase in frequent (OR 3.2; 95% CI: 2.0-3.0) and infrequent wheezing (OR 4.3; 95% CI: 2.2-8.7) at six years of age in children with RSV LRTI during the first three years of life, but this association became non-significant by age 13 years (144). It is unknown why pulmonary sequelae or wheezing symptoms did not persist into adolescence, but it is possibly due to the increased airway diameter from the subsequent growth of the airways, and suggests that the symptoms at six years of age in children with RSV LRTI during the first three years of life are not due to an increase in asthma prevalence, as it is unlikely that the symptoms would then not diminish with time.

1.9.2 RSV and asthma

Less well established than the association between RSV and wheezing is the relationship between RSV and asthma, which ideally requires the objective assessment of pulmonary function testing and bronchial hyperreactivity. Asthma is the most common non-communicable disease in children worldwide (145), both in LMIC and HIC (146, 147). Children in LMIC with asthma are more likely to have severe symptoms and are less likely to be diagnosed timeously, or have access to appropriate medical management. Consequently >80% of global asthma related mortality occurs in LMIC, highlighting the importance of evaluating the role that RSV LRTI may play in the development of asthma.

There has been a few well conducted longitudinal cohort studies that showed an increase in asthma following RSV LRTI in infancy. In a prospective longitudinal case-control study examining infants with RSV LRTI hospitalization from Sweden, Sigurs et al. reported data at several time-points; i.e.: three years (148), 7.5 years (149), 13 years (150) and 18 years (151). At three and 7.5 years there was an increase in the incidence of any wheezing, wheezing in the last 12 months and physician diagnosed asthma, as well as allergic sensitisation in the cases. At the 13-year follow-up, there was also an increase in recurrent wheezing and physician diagnosed asthma, as well as allergic rhino-conjunctivitis and allergic sensitisation to common aeroallergens in cases. At 18 years, there was an increase in the prevalence of asthma, recurrent wheeze,

clinical allergy and allergic sensitisation in cases. Spirometry measurements (forced expiratory volume in one second (FEV_1), FEV_1/ forced vital capacity (FVC) and forced expiratory flow at 25–75% of forced vital capacity (FEF_{25-75}) indicated obstructive airways disease, as well as a bronchodilator response in the cases providing some objective evidence of asthma after RSV LRTI hospitalization.

A large population based case-control study from Scotland (740 418 children born in the National Health Service between 1996 and 2011), reported a three-fold increase in risk of asthma hospitalization and a two-fold increase in asthma medication usage in the first 18 years of life after admission for RSV LRTI during the first two years of life (152). RSV LRTI hospitalization was the most significant risk factor for future asthma hospitalizations (OR 2.80; 95% CI: 2.60-3.02) and confirmed asthma (OR 1.85; 95% CI: 1.78-1.92) in this cohort. This study however did not answer the question of whether the RSV LRTI hospitalization was the cause of the asthma in later years or whether the children who had a genetic or environmental predisposition developed asthma.

In a prospective birth cohort study investigating term infants hospitalized for RSV-associated bronchiolitis, it was reported that at six years of age, cases were more likely to have current wheeze (aOR 3.2; 95% CI: 1.2-8.1) and physician diagnosed asthma (with concurrent symptoms or medication use) (aOR 3.1; 95% CI: 1.3-7.5) than healthy term infants controls (153). This study included objective pulmonary function measurements and reported a decrease in FEV_1% in cases. Measurements of bronchial reactivity with their pulmonary function tests were however not performed and therefore questions the diagnosis of asthma. In a large case-control study from Greece, infants hospitalized for RSV-associated bronchiolitis were more likely, than the general Greek population, to be diagnosed with asthma at 7.5 years (57.1% vs 7-10%)(154). Similar to the previous study, no bronchodilator response test was performed. A large population based longitudinal cohort study, the Avon Longitudinal Study of Parents and Children, reported an increase of wheezing at 30-42 (28.1% vs 13.1% in controls) and 69-81 (22.6% vs 9.6%) months of age in those with RSV-LRTI before two years of age (155). They also reported an increase in the cumulative incidence of asthma between cases and controls at seven years age (38.4% vs 20.1%), but no objective measurements were performed.

Importantly, other factors may account for an asthma diagnosis in children that also had RSV. In a cohort of 206 infants with severe RSV-associated bronchiolitis, 48% had physician diagnosed

asthma at seven years of age (107). However, maternal asthma (OR 5.2; 95% CI: 1.7-15.9), aeroallergen sensitivity at three years (OR 10.7; 95% CI: 2.1-55.0), and recurrent wheezing until age three (OR 7.3; CI: 1.2-43.3) were associated with the increased risk of physician diagnosed asthma in this study that may indicate that these children were more predisposed to an atopic disposition and not necessarily due to the RSV LRTI. Furthermore, in a retrospective case-control study from rural Alaska, RSV LRTI associated hospitalization before two years of age was associated with an increase in physician diagnosed asthma by four years of age, but these associations were no longer significant by five years (156). Similarly to the above study, cases were more likely to have had allergies, eczema, and a positive family history of asthma, and this raises the concern of how physicians diagnosed asthma, and conclusion reached by the study.

In contrast to the above studies, Kotaniemi-Syrjanen, found that RSV identification during the index wheezing episode was not associated with the development of asthma at seven years of age, whereas blood eosinophilia, atopic dermatitis, elevated serum immunoglobulin E (IgE), and a history of earlier episodes of wheezing was associated with the development of asthma (157). Subsequently, reporting 11 year follow-up data from the same birth cohort, the highest risk for developing asthma in this group was in atopic infants; with atopic dermatitis and the presence of specific IgE to inhalant allergens being early predictors of asthma (158). The risk, however, for developing asthma was five-fold increased after RSV-associated wheezing and 10-fold increased after Rhinovirus-associated wheezing. At the 20 year follow-up of the cohort, RSV was found not to be a risk factor for asthma, but was associated with lung function abnormalities, namely a decreased FEV_1% and maximal expiratory flow at 25% of FVC (MEF_{25}), with no difference between cases and controls in bronchial reactivity (159). This well-designed long-term follow-up study is likely the most important study investigating the long-term pulmonary effects of RSV-associated hospitalization during infancy. It combines long-term questionnaire based follow-up over multiple time points with a definitive objective measurement of pulmonary function testing including bronchodilator responsiveness.

The lack of association between asthma and RSV is also supported by a study examining 37 monozygotic twin sets discordant for admission for severe RSV-associated bronchiolitis during infancy. Poorisrisak found no difference in asthma prevalence, asthma medication use, allergen sensitisation, or pulmonary function results at 7.6 years (160). Due to the nature of this study (monozygotic twins), which naturally controls for genetic factors and to a certain extent for

environmental factors, it casts doubt that RSV alone causes an increase in asthma and that multiple factors are likely involved in the pathogenesis of asthma developing in children.

Therefore, although the association between RSV LRTI in the early years of childhood and subsequent pulmonary sequelae, especially wheezing, seems well documented, the question whether RSV causes asthma is still mostly unanswered, with few studies properly designed to account for the multiple variables that are involved in the pathogenesis of asthma.

1.9.3 RSV and pulmonary function testing

A growing number of studies have used pulmonary function tests (PFT) to examine the association between RSV infection during early childhood and pulmonary sequelae later in life. This provides an objective assessment of airway function rather than the subjective reporting associated with parental or patient based questionnaires. Paediatric lung function testing techniques, especially spirometry, are well established in older children (>6 years of age) (161-163). Spirometry is, however, difficult to perform in children younger than six years of age due to the requirement of a forced coordinated breathing maneuver. Criteria for acceptability are not easily achieved in younger children, and this is indirectly associated with their age (164).

This has led to the utilization of other pulmonary function techniques, including forced oscillation technique (FOT), multiple breath inert gas washout technique (MBW) and tidal breath flow volume loops (TBFVL). These tests are now recognized as acceptable tests to be performed in preschool children and infants (161). Through multi-centre collaborations these techniques are now also standardized and reference values for these age groups are available (161, 165-168).

Previous systematic reviews have analyzed the association between RSV LRTI and pulmonary sequelae through parental questionnaires or physician diagnosis of wheezing (169, 170). One systematic review reported an increased risk of impaired pulmonary function following RSV LRTI in early childhood (170). This review however, had some limitations: only studies from January 1985 – December 2015 were included, studies from outside the United States, Canada and Europe were excluded, assessments only included children under 18 years of age, and only studies that reported a positive association between RSV LRTI and subsequent pulmonary function abnormalities were mentioned.

For these reasons, we pursued a systematic review, which was performed in accordance with a published protocol following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses-Protocol (PRISMA-P) guideline of 2015 (171, 172). This systematic review examined the association between RSV and subsequent pulmonary function sequelae, measured through different techniques of pulmonary function testing, following proven RSV LRTI in children within the first three years of life (174) (The published paper has been added as Appendix 1).

1.9.3.1 Systematic Review Methodology

The following databases were searched: PubMed, Scopus, Cochrane Library, and the World Health Organization Global Index Medicus. In addition, ClinicalTrials.gov and Cochrane Central Register of Controlled Trials (Central) for ongoing or unpublished trials were assessed, of which there were none. The following PubMed search strategy was used: (RSV[All Fields] OR ("respiratory syncytial viruses"[MeSH Terms] OR ("respiratory"[All Fields] AND syncytial"[All Fields] AND "viruses"[All Fields]) OR ("respiratory syncytial viruses"[All Fields]) OR ("respiratory"[All Fields] AND "syncytial"[All Fields] AND "virus"[All Fields]) OR "respiratory syncytial virus"[All Fields] OR ("bronchiolitis"[MeSH Terms] OR "bronchiolitis"[All Fields]) AND "respiratory function tests"[MeSH Terms] OR ("respiratory"[All Fields] AND "function"[All Fields] AND "tests"[All Fields]) OR ("respiratory function tests"[All Fields]).

All interventional (randomized control trials, cluster-randomized control trials, and non-randomized control trials) and observational studies (cohort studies, case-control studies, and cross-sectional studies) that had the end-point outcome of PFTs after a proven RSV LRTI in the first three years of life were included. There were no limits placed on the year of publication and studies published up until the date that the final search was performed were included. The search was, however, limited to human studies published in English, Portuguese, Spanish, and German.

PFTs could include spirometry, body plethysmography, gas washout techniques, FOT, and thoraco-abdominal compression techniques. Comparator groups were children that did not have a LRTI or had a LRTI caused by a different organism. Secondary outcome measurements were a PFT measurement in children who had a documented RSV LRTI and an underlying medical

condition (e.g. premature birth, low birthweight, Trisomy 21); and pulmonary sequelae stratified by severity of RSV LRTI, age at time of RSV LRTI, and socioeconomic status.

In reporting the lung function parameters, the broad groups of abnormal lung function parameters were defined as follows: obstructive airways disease when there was a decrease in FEV_1 or any of the other indices of flow, for example, FEF_{25-75}, restrictive lung disease when there was a decrease in the FVC and a normal FEV_1/FVC ratio and mixed lung disease when there was a decrease in FVC with a decrease in the FEV_1/FVC ratio.

1.9.3.2 Systematic Review Results

The final analysis included 31 studies (108, 143, 144, 150, 151, 153, 154, 159, 173-195) (An abbreviated summary of the studies included in the review are given in Table 1.1). Spirometry was performed in 29 studies, oscillometry in five, and one each of body plethysmography, interrupter technique, sulfur hexafluoride (SF6) multiple-breath washout, helium gas dilution technique, single-breath nitrogen washout, and parasternal intercostal electromyography.

Thirteen (45%) of 29 studies using spirometry, reported no association between RSV LRTI and pulmonary function sequelae between five and 19 years of age. The remaining 16 (55%) studies reported abnormal spirometry between six to 31 years of age; including 12 (41%) that reported obstructive airways disease between seven to 30 years of age, three restrictive lung disease, and one (3%) mixed lung disease. Twenty-seven studies reported specifically on FEV_1, of which nine (33%) reported an abnormal FEV_1 in cases with prior RSV LRTI (Table 1.2). Five of these reported a lower baseline FEV_1 at six to 18 years of age in those with previous RSV LRTI, three reported a decreased pre- and post- bronchodilator (BD) FEV_1 at seven to 30 years of age, and one study reported a decreased pre-BD FEV_1 with no difference in post-BD FEV_1 at 11 years age.

There were three long-term longitudinal cohort studies reporting spirometry results on RSV LRTI associated pulmonary sequelae at different time points. In a Finnish birth cohort (1981-1982), there was no association between RSV LRTI occurring within two years of birth and subsequent abnormal pulmonary function at 8 to 10 years, although airflow limitation (decreased FEV_1/FVC and MEF_{25}) was evident at 20 years of age and irreversible airflow obstruction at 30 years of age

(decreased FEV_1 and FEV_1/FVC with no BDR). In another Finnish study, children hospitalized for RSV bronchiolitis at less than two years of age (1992-1993) and compared to those admitted for non-RSV bronchiolitis, had mixed restrictive and obstructive lung disease (decreased FVC and FEV_1) at 12 years of age. At 19 years of age, these RSV bronchiolitis cases had minimal lung function abnormalities (decreased pre-BD FVC) when compared to matched controls never admitted for LRTI during infancy. Lastly, in Sweden, cases hospitalized with RSV-associated bronchiolitis had irreversible airflow obstruction (decreased FEV_1/FVC, forced expiratory flow at 75% of FVC (FEF_{75}), pre- and post-BD with no difference in BDR between groups) at 13 and 18 years (decreased FEV_1, FEV_1/FVC, and FEF_{25-75}) when compared to matched controls. These studies, even though only including small numbers of cases, but with excellent long-term follow-up patient retention, are some of the strongest studies available to date with regards to the pulmonary sequelae of RSV infection in infancy, due to the duration of follow-up in these very well defined populations. The main finding in two of these studies was obstructive airways disease with no bronchodilator response, or irreversible small airway obstruction.

Many of the studies included in this review enrolled small numbers of participants; consequently, any inference from these studies would be modest. Only five studies enrolled more than 100 patients and even though they included participants at a wide range of ages (six to 11 years), used different PFTs and reported different pulmonary function indices, their results reflect the overall findings of the review; that of varying abnormal pulmonary function favoring obstructive airways disease (143, 144, 153, 154, 188). The results of the four studies reporting on longitudinal cohorts also mirrored these results with varying abnormal pulmonary function favoring obstructive lung disease (108, 150, 151, 159, 173, 174, 182, 184, 191).

Abnormal pulmonary function also increased with older age at testing, with 77% (10 of 13) of studies testing individuals over 10 years of age reporting abnormal spirometry results, compared to 38% (6 of 16) of studies performed in children under 10 years. This was highlighted in two Finnish cohorts that reported abnormal pulmonary function results at 20 and 30 years but normal pulmonary function at 8 to 10 years and no difference in oscillometry indices at seven years, but obstructive airways disease with spirometry at 13 years, possibly indicating damage to and subsequent arrest of airway growth more so than lung parenchymal damage (159, 174, 182, 184, 191).

Table 1.1: Characteristics of studies included in the systematic review

Study ID (author and year)	Study design	Country of study	Case group	Type of PFT*
Backman et al. 2018 (173)	Case-control study nested in a cohort study	Finland	Children <2 years of age admitted with RSV† LRTI‡ assessed at 20 years	Spirometry with BDR§ (salbutamol 400ug)
Backman et al. 2014 (174)	Case-control study nested in a cohort study	Finland	Children <2 years of age admitted with RSV LRTI assessed at 30 years of age	Spirometry with BDR (salbutamol 400ug)
Broughton et al. 2007 (175)	Birth cohort study	United Kingdom	Premature infants with RSV LRTI assessed at 1 year corrected age	Body plethysmography Helium gas dilution
Carbonell-Estrany et al. 2015 (176)	Case-control study nested in a cohort study	Spain	Premature infants admitted with RSV LRTI assessed at 6 years of age	Spirometry
Cassimos et al. 2006 (154)	Case-control study	Greece	Infants admitted with RSV bronchiolitis assessed at 7 years of age	Spirometry
Fjaerli et al. 2005 (177)	Case-control study	Norway	Infants admitted with RSV bronchiolitis assessed at 7 years of age	Spirometry with BDR (salbutamol 200ug)
Greenough et al. 2009 (178)	Case-control study nested in a cohort study	United Kingdom	Children <2 years of age with prematurity and BPD admitted with RSV LRTI assessed at 10.5 years of age	Spirometry with challenge (cold air) and BDR (salbutamol 200ug)
Guilbert et al. 2011 (179)	Birth cohort study	USA	Children <3 years with RSV LRTI assessed at 8 years of age	Spirometry with BDR (salbutamol 200ug) Oscillometry (IOS)‖
Hall et al. 1984 (180)	Cohort study	USA	Children <2 years of age admitted with RSV LRTI assessed at 7 years of age	Spirometry
Hyvarinen et al. 2007 (108)	Cohort study	Finland	Children <2 years of age admitted with RSV bronchiolitis assessed at 12 years of age	Spirometry with challenge (metacholine and exercise)
Juntti et al. 2003 (181)	Case-control study	Finland	Infants admitted with RSV LRTI assessed at 8 years of age	Spirometry Oscillometry (IOS) with BDR (Unknown)
Korppi et al. 1994 (182)	Case-control study nested in a cohort study	Finland	Children <2 years of age admitted with RSV LRTI assessed at 8-10 years	Spirometry

Table 1.1 cont.: Characteristics of studies included in the systematic review

Study ID (author and year)	**Study design**	**Country of study**	**Case group**	**Type of PFT***
Korppi et al. 2004 (159)	Case-control study nested in a cohort study	Finland	Children <2 years of age admitted with RSV LRTI assessed at 18-20 years	Spirometry and challenge (metacholine)
Krilov et al. 1997 (183)	Cohort study	USA	Infants who participated in a trial of ribavirin during RSV LRTI admission assessed at 5-6 years of age	Spirometry
Lauhkonen et al. 2015 (184)	Cohort study	Finland	Infants <6 months admitted with RSV bronchiolitis assessed at 6-7 years of age	Oscillometry (IOS) with challenge (exercise) and BDR (salbutalmol 300mg)
Long et al. 1997 (185)	Cohort study	USA	Infants who participated in a trial of ribavirin during RSV LRTI admission assessed at 7-10 years of age	Spirometry
MacBean et al. 2018 (186)	Cohort study	United Kingdom	Premature infants admitted with RSV LRTI assessed at 6 years of age	Spirometry with challenge (metacholine) Oscillometry (IOS) Parasternal intercostal EMG**
Mikalsen et al. 2012 (187)	Case-control study	Norway	Infants admitted with RSV bronchiolitis assessed at 11 years of age	Spirometry with challenge (metacholine)
Mok et al. 1982 (188)	Case-control study	United Kingdom	Infants admitted with RSV LRTI assessed at 7 years	Spirometry Oscillometry (FOT)††
Piippo-Savolainen et al. 2007 (189)	Case-control study	Finland	Children <2 years of age admitted with RSV bronchiolitis assessed at 19 years of age	Spirometry with challenge (metacholine)

Table 1.1 cont.: Characteristics of studies included in the systematic review

Study ID (author and year)	Study design	Country of study	Case group	Type of PFT*
Poulsen et al. 2006 (190)	Case-control study	Guinea-Bissau	Infants with RSV LRTI assessed at 7 years of age	Spirometry
Pullan et al. 1982 (143)	Case-control study	United Kingdom	Infants admitted with RSV LRTI assessed at 10 years of age	Spirometry with challenge (histamine) Single breath nitrogen washout
Riikonen et al. 2018 (191)	Case-control study	Finland	Infants <6 months of age admitted for RSV bronchiolitis assessed at 12 years of age	Spirometry with BDR (salbutamol 400ug)
Rodriguez et al. 1999 (192)	Cohort study	USA	Infants who participated in a trial of ribavirin during RSV bronchiolitis admission assessed at 8 years of age	Spirometry with challenge (metacholine)
Sigurs et al. 2005 (150)	Cohort study	Sweden	Infants admitted with RSV bronchiolitis assessed at 13 years of age	Spirometry with challenge (dry air)
Sigurs et al. 2010 (151)	Cohort study	Sweden	Infants admitted with RSV LRTI assessed at 18 years of age	Spirometry with challenge (cold air) Multiple breath washout
Sims et al. 1978 (193)	Case-control study	United Kingdom	Infants admitted with RSV bronchiolitis assessed at 8 years of age	Spirometry
Sly et al. 1989 (194)	Cohort study	Australia	Infants admitted for RSV bronchiolitis assessed at 6 years of age	Spirometry with challenge (histamine)
Stein et al. 1999 (144)	Birth cohort study	USA	Children <3 years of age with RSV LRTI assessed at 11 years of age	Spirometry with BDR (salbutamol 180ug)
Welliver et al. 1993 (195)	Cohort study	USA	Infants admitted for RSV bronchiolitis assessed at 7-8 years of age	Spirometry with challenge (metacholine
Zomer-Kooijker et al. 2014 (153)	Case-control study nested in a cohort study	The Netherlands	Infants admitted with RSV LRTI assessed at 6 years of age	Spirometry Interupter technique

*PFT: pulmonary function test, †RSV: Respiratory syncytial virus, ‡LRTI: lower respiratory tract infection, §BDR: bronchodilator response, ||IOS: impulse oscillometry, **EMG: electromyography, ††FOT: forced oscillation technique

None of the five studies reporting oscillometry data detected an increased resistance in children who had a RSV LRTI (179, 181, 184, 186, 188) (Table 1.3). Body plethysmography was performed in one study where an increased airway resistance at one year of age in infants born at <32weeks gestational age (GA) admitted with a RSV LRTI was reported (175). Using the interrupter technique Zomer-Kooijker et al. reported an increase in resistance at six years age in infants admitted for RSV LRTI (153).

1.9.3.3 Systematic Review Discussion

The biggest challenge in interpreting the data from the studies included in this systematic review was the heterogeneity in the methodology of different studies. This included lack of standardization regarding study design, time frames for testing, type of tests performed, end-points measured and reporting methods making comparisons between the studies difficult. All of these factors mentioned above and expanded on below, have the potential to increase the risk of bias in the individual studies. Multiple different methods of pulmonary function testing were used across studies and we were unable to compare results across different testing modalities. Although spirometry was the one test used most often, many different pulmonary function indices were reported ranging from the reporting of only an abnormal FEV_1 to multiple abnormal indices as well as different reporting units for the various indices, such as absolute numbers (liters), standard deviation, and percentage predicted. There was a lot of selective reporting of results and having all the data from the studies available would have added great value. Different reference values for the pulmonary function tests were used.

Most of the studies were from Europe and the United States and, therefore, not generalizable across other populations and ethnic groups. Initial recruitment of cases ranged from six months to three years of age and the ages at which the participants underwent PFTs ranged from one to 30 years of age. In the rapidly developing respiratory system of the preschool child, there is a vast difference between the lungs of children at these ages and the studies at the extremes of this age spectrum cannot be compared (196).

Table 1.2: Forced expiratory volume in one second data from included studies

Study ID	Age at RSV* infection (years)	Age of testing (years)	Cases		Controls		p-value[a] / Odds Ratio	Results interpretation
			Pre-BD†	Post-BD	Pre-BD	Post-BD		
Zomer-Kooijker et al. (153)	< 1	6	FEV_1 93.3 (12.19)[c]		FEV_1 100.27 (13.90)[c]		<0.001	Restrictive lung disease (Normal FEV_1/FVC ratio) after RSV LRTI
Carbonell-Estrany et al. (176)	< 1	6	FEV_1 -0.39 (1.10)[d]		FEV_1 -0.29 (1,03)[d]		0.532	No association between RSV LRTI and abnormal lung function
Sly et al. (194)	< 1	6	FEV_1 106 (18)[c]					No association between RSV bronchiolitis and abnormal lung function
MacBean et al. (186)	< 1	6	FEV_1 0.14 (-1.04,1.33)[e]	FEV_1 -0.23 (-8.79,8.32)[e]	FEV_1 0.02 (-0.45,0.49)[e]	FEV_1 4.16 (-0.01,8.32)[e]		No association between RSV LRTI and abnormal lung function
Guilbert et al. (179)	< 3	6-8	FEV_1 100 (2)[f]	FEV_1 109 (2)[f]	FEV_1 101 (1)[f]	FEV_1 107 (1)[f]	0.97	No association between RSV wheezing illness and abnormal lung function
Poulsen et al. (190)	< 1	7	FEV_1 41%[g]		FEV_1 65%[g]		0.28 (0.10, 0.79)	Obstructive airways disease after RSV LRTI
Hall et al. (180)	< 2	7	FEV_1 94.3 (7.9)[c]					Obstructive airways disease after RSV LRTI
Mok et al. (188)	< 1	7	FEV_1 91.3 (13.5)[c]		FEV_1 91.2 (12.2)[c]		1.0	No association between RSV LRTI and abnormal lung function
Cassimos et al. (154)	< 1	7-8	FEV_1 90.0 (11.6)[c]		FEV_1 102.8 (11.6)[c]		< 0.05	Obstructive airways disease after RSV LRTI
Welliver et al. (195)	< 1	7-8	FEV_1 98 (3)[f]					Normal lung function after RSV bronchiolitis

Table 1.2 cont.: Forced expiratory volume in one second data from included studies

Study ID	Age at RSV infection (years)	Age of testing (years)	Cases		Controls		p-value / Odds Ratio	Results interpretation
			Pre-BD	Post-BD	Pre-BD	Post-BD		
Fjaerli et al. (177)	< 1	8	FEV_1 1.51 (0.05,0.23)[h]	FEV_1 1.59 (0.03,0.20)[h]	FEV_1 1.64[h]	FEV_1 1.70[h]		Obstructive airways disease after RSV bronchiolitis
Juntti et al. (181)	< 1	8	FEV_1 102.9 (13.9)[c]		FEV_1 102.9 (13.9)[c]			No association between RSV LRTI and abnormal lung function
Rodriguez et al. (192)	< 1	8	FEV_1 87.6 (4.0)[f]		FEV_1 79.2 (3.0)[f]		0.16	Normal lung function after RSV bronchiolitis
Korppi et al. (182)	< 2	8-10	FEV_1 1.60 (0.30)[i]		FEV_1 1.71 (0.33)[i]		0.266	No association between RSV LRTI and abnormal lung function
Long et al. (185)	< 1	10	FEV_1 95.4[j]					Normal lung function after RSV LRTI
Pullan et al. (143)	< 1	10	FEV_1 92.8[j]		FEV_1 101.5[j]		<0.001	Mixed lung disease (Decreased FVC and FEV_1, decreased FEV_1/FVC ratio)) after RSV LRTI
Stein et al. (144)	< 3	11	FEV_1 2.11 (2.05,2.15)[k]	FEV_1 2.26 (1.70,2.90)[k]	FEV_1 2.22 (2.18,2.25)[k]	FEV_1 2.31 (1.70,2.99)[k]	<0.001	Obstructive airways disease after RSV LRTI
Mikalsen et al. (187)	< 1	11	FEV_1 96.1 (93.9, 98.2)[l]		FEV_1 99.1 (96.7, 101.4)[l]		0.061	No association between RSV LRTI and abnormal lung function

Table 1.2 cont.: Forced expiratory volume in one second data from included studies

Study ID	Age at RSV infection (years)	Age of testing (years)	Cases		Controls		p-value / Odds Ratio	Results interpretation
			Pre-BD	Post-BD	Pre-BD	Post-BD		
Riikonen et al. (191)	< 0.5	12	FEV_1 17 (27.0%)[m]	FEV_1 12 (19.0%)[m]	Not reported	Not reported	2.9 (1.2, 6.7)	Obstructive airways disease after RSV bronchiolitis
Hyvarinen et al. (108)			FEV_1 98.35 (6.53)[c]		FEV_1 92.91 (8.70)[c]		0.033	Restrictive lung disease (Normal FEV1/FVC ratio) after RSV bronchiolitis
Sigurs et al. (150)	< 1	13	FEV_1 90.8 (10.3)[c]	FEV_1 94.0 (10.5)[c]	FEV_1 93.2 (10.3)[c]	FEV_1 95.8 (10.6)[c]	0.211	Obstructive airways disease (Normal FEV_1, decreased FEV_1/FVC) after RSV bronchiolitis
Sigurs et al. (151)	< 1	18	FEV_1 -0.28 (0.93)[d]		FEV_1 0.11 (1.08)[d]		<0.05	Obstructive airways disease after RSV LRTI
Korppi et al. (159)	< 12	18-20	FEV_1 100 (95, 105)[l]		FEV_1 104 (100, 108)[l]		0.176	Obstructive airways disease after RSV LRTI (Normal FEV_1, decreased FEV_1/FVC)
Backman et al. (173)	< 2	19	FEV_1 90 (84, 96)[l]	FEV_1 93 (87, 99)[l]	FEV_1 95 (92, 98)[l]	FEV_1 98 (95, 101)[l]	0.139	Restrictive lung disease after RSV LRTI (decreased FVC)
Piippo-Savolainen et al. (189)	< 2	19	FEV_1 100 (94, 107)[l]		FEV_1 95 (90, 101)[l]		0.243	No association between RSV bronchiolitis and abnormal lung function
Backman et al. (174)	< 2	30	FEV_1 86 (81, 91)[l]	FEV_1 90 (85, 96)[l]	FEV_1 96 (94, 98)[l]	FEV_1 100 (98, 101)[l]	<0.001	Obstructive airways disease after RSV LRTI

*RSV: Respiratory syncytial virus; †BD: bronchodilator; ‡FEV_1: forced expiratory volume in one second; §LRTI: lower respiratory tract infection; [a] P-value: comparing pre-bronchodilator FEV_1 between cases and controls; [b]Percentage predicted: Median (Interquartile range); [c]Percentage predicted: Mean (Standard deviation); [d]Z-scores: Mean (Standard deviation); [e]Z-scores: Median (Interquartile range); [f]Percentage predicted: Mean (Standard error); [g]Percentage higher than the median (adjusted odds

ratio); [h]Absolute values (liters): Mean (95% Confidence interval of difference between means); [i]Absolute value (liters): Mean (Standard deviation); [j]Percentage predicted: Mean; [k]Absolute values (liters): Mean (95% Confidence interval); [l]Percentage predicted: Mean (95% Confidence interval); [m]Number (%) under normal percentage predicted: Adjusted odds ratio (95% Confidence interval)

Table 1.3: Pulmonary function testing data (non-spirometry)

Study ID	Age of RSV* (Years)	Age of testing (Years)	Test	Cases		Controls		p-value[a]	Interpretation
				Pre-BD†	Post-BD	Pre-BD	Post-BD		
Broughton et al. (175)	< 1	1	Body plethysmo-graphy	$FRC‡_{(Pleth)}$: 300.6 (57.4)[b], $FRC_{(Pleth)}$: 30.7 (6.0)[c], $R_{(aw)}$§: 2.59 (0.76)[d]		$FRC_{(Pleth)}$: 295.7 (47.0)[b], $FRC_{(Pleth)}$: 30.5 (4.4)[c], $R_{(aw)}$: 1.84 (0.64)[d]		0.725 0.854 0.002	Infants born <32 wGA¶ with RSV LRTI** had increased airway resistance at one year
Broughton et al. (175)	< 1	1	Helium gas dilution technique	$FRC_{(He)}$: 243.6 (44.3)[b], $FRC_{(He)}$: 24.8 (3.7)[c]		$FRC_{(He)}$: 243.6 (44.0)[b], $FRC_{(He)}$: 24.9 (3.7)[c]		0.999 0.931	
MacBean et al. (186)	< 1	6	Oscillometry	R5†† 0.41 (-0.69,1.50)[e], R20‡‡ 0.06 (-1.22,1.34)[e]		R5 0.58 (0.20,0.95)[e], R20 0.11 (-0.31,0.54)[e]			No association between RSV LRTI and abnormal oscillometry at 6 years
Lauhkonen et al. (184)	< 0.5	6	Oscillometry	R5 0.0228 (0.87153)[f], R20 -0.7772 (0.94560)[f], X5¶¶ -0.73175 (1.058865)[f],	R5 -1.6816 (0.77749)[f], R20 -1.8891 (0.96826)[f], X5 0.3300 (0.71457)[f],	R5 -0.2524 (1.28958)[f], R20 -1.1937 (1.31448)[f], X5 -0.74927 (1.441022)[f],	R5 -1.7715 (1.22896)[f], R20 -2.1817 (1.40975)[f], X5 0.3323 (0.65439)[f],	0.1988 0.0644 0.9435 0.6500 0.2144 0.0354	No association between RSV + and RSV - bronchiolitis and abnormal oscillometry at 6 years

Table 1.3 cont.: Pulmonary function testing data (non-spirometry)

Study ID	Age of RSV (Years)	Age of testing (Years)	Test	Cases		Controls		p-value	Interpretation
				Pre-BD	Post-BD	Pre-BD	Post-BD		
Zomer-Kooijker et al. (153)	< 1	6	Interrupter technique	R_{int}†††: 0.76 (0.23)[d], R_{int}(%): 124.72 (35.69)[d]		R_{int}: 0.66 (0.17)[d], R_{int}(%): 112.73 (31.88)d		0.002 0.002	Infants admitted for RSV LRTI had increased airway resistance at 6 years
Guilbert et al. (179)	< 3	6-8	Oscillometry	R5 0.90 (0.01) (-0.06,0.04)[h], R10‡‡‡ 0.74 (0.02) (-0.01,0.07)[h], R5-10 0.16 (0.01) (-0.05,0.00)[h], R20 0.56 (0.01) (-0.02,0.04)[h], X5 0.37 (0.01) (-0.05,0.01)[h], AX§§§ 2.77 (0.81,1.03)[h]		R5 0.91 (0.02)[h], R10 0.71 (0.01)[h], R5-10 0.19 (0.01)[h], R20 0.55 (0.01)[h], X5 0.39 (0.01)[h], AX 3.04[h]		0.64 0.16 0.04 0.62 0.12 0.13	No association between RSV wheezing illness and abnormal oscillometry at 6-8 years
					R5 0.71 (0.01) (-0.06,0.03)[h], R10 0.60 (0.02) (-0.03,0.04)[h], R5-10 0.10 (0.01) (-0.04,0.00)[h], R20 0.51 (0.01) (-0.02,0.05)[h], X5 0.29 (0.01) (-0.04,0.01)[h],		R5 0.72 (0.02)[h], R10 0.60 (0.02)[h], R5-10 0.12 (0.01)[h], R20 0.49 (0.01)[h], X5 0.31 (0.01)[h],	0.62 0.66 0.04 0.40 0.17	

Table 1.3 cont.: Pulmonary function testing data (non-spirometry)

Study ID	Age of RSV (Years)	Age of testing (Years)	Test	Cases		Controls		p-value	Interpretation
				Pre-BD	Post-BD	Pre-BD	Post-BD		
Mok et al. (188)	< 1	7	Oscillometry	Rt¶¶¶ 0.60 (0.17)[i]		Rt 0.56 (0.18)[i]		1.0	No association between RSV LRTI and abnormal oscillometry at 7 years
Juntti et al. (181)	< 1	8	Oscillometry	R5 85.0 (19.1)[I], R20 96.4 (18.2)[i]	R5(%) 15.3 (12.8)[I], R20(%) 14.7 (10.9)[i]	R5 86.5 (21.2)[I], R20 96.4 (18.2)[i]	R5(%) 14.6 (16.6)[I], R20(%) 11.5 (15.0)[i]		No association between RSV LRTI and oscillometry at 8 years
Pullan et al. (143)	< 1	10	Single breath nitrogen wash-out	Mean phase III slope: 1.24 (0.62)[i]		Mean phase III slope: 1.35 (0.49)[i]			No association between RSV LRTI and abnormal single breath nitrogen washout at 10 years
Sigurs et al. (151)	< 1	18	Multiple breath washout (SF6)****	LCI 6.63 (0.52) (-0.21, 0.13)[h]		LCI†††† 6.59 (0.43)[h]			No association between RSV LRTI and LCI at 18 years

*RSV: Respiratory syncytial virus; †BD: bronchodilator; ‡FRC: functional residual capacity; §$R_{(aw)}$: airway resistance; ¶wGA: weeks gestational age; **LRTI: lower respiratory tract infection; ††R5: resistance at 5Hz; ‡‡R20: resistance at 20Hz; §§Z5: impedance at 5Hz: ¶¶X5: reactance at 5Hz, ***Fres: resonant frequency; †††R_{int}: resistance measured via interrupter technique; ‡‡‡R10: resistance at 10Hz; §§§AX: area under the reactance curve; ¶¶¶R_t: total resistance; ****SF6: Sulphur hexafluoride; ††††LCI: lung clearance index. [a]P-value: comparing values between cases and controls, [b]Absolute values (milliliter): Mean (standard deviation), [c]Absolute values (milliliter/kilogram): Mean (standard deviation), [d]Absolute values (kilopascal/liter/second): Mean (standard deviation), [e]Z-score: Median (interquartile range), [f]Z-score: Mean (standard deviation), [g]Percentage predicted: Mean (standard deviation), [h]Mean (standard deviation)(95% confidence interval), [i]Mean (standard deviation)

The pulmonary function sequelae acquired after a RSV LRTI at these different ages could potentially manifest very differently. Furthermore it is common to start investigating pulmonary function in children at around six to seven years of age and then to use spirometry as the main investigational tool. This is due to the inherent difficulties young children experience trying to perform this test (162). This however, provides no information on the very important time period between the RSV LRTI and this later age of testing. This is generally seen as the time period where asthma manifests in children. More longitudinal cohort data is needed with the aid of additional age-appropriate PFTs, for example, forced oscillation technique, multiple-breath inert gas washout, and tidal breath follow volume loops, to be able to delineate the progression of lung disease during this crucial time period. Different definitions (bronchiolitis or LRTI) for inclusion of participants into the studies were used as well as different levels of severity of the initial RSV infection. Very few studies reported full detailed demographic characteristics that are relevant to pulmonary function outcomes. Information regarding participants' sex, ethnic background, birthweight, GA at birth, underlying medical conditions, including HIV infection status, age at RSV infection, duration of admission and level of medical intervention, were often not reported. These are important confounders and determinants of severity and outcome after RSV LRTI and should be controlled for and reported. The majority of the studies were from HICs with only one study from outside this income group (190). This would make the results from this review non-generalizable to most countries around the world, and particularly for LMICs where the burden of severe RSV LRTI is greatest.

1.9.3.4 Studies undertaken after the systematic review

A few studies from South Africa have explored the feasibility of infant and childhood pulmonary function testing in a LMIC, and data from the Drakenstein Child Health Study, has been published (145, 197-200). The authors have reported on the effect of early LRTI on pulmonary function at one year, and concluded that early LRTI is associated with an increased respiratory rate, and that subsequent LRTIs resulted in a further increase in the respiratory rate, a decrease in tidal volume, and an increase in the LCI (198). Data from the two year follow-up, including association between RSV-associated LRTI and two year pulmonary function has recently been published (19). They reported that RSV LRTI, as well as hospitalization for all-cause LRTI, were

associated with recurrent wheezing. LRTI or recurrent LRTI was also associated with impaired lung function, independent of whether the LRTI was associated with RSV.

1.10. Justification for conducting research study

There is a paucity of studies describing pulmonary sequelae of LRTI, especially following RSV LRTI, and only one study from Africa (19, 190). As highlighted in our systematic review, the majority of studies were conducted in children six years of age or older, and used spirometry for testing purposes (201). There was also significant heterogeneity between studies describing the long-term pulmonary effects of RSV LRTI; the main findings was the presence of obstructive airways disease with no bronchial hyper-responsiveness.

RSV LRTI affects the developing lung during the alveolar stage and may potentially lead to an arrest or decrease in growth velocity of the small airways, or a narrow airway diameter that presents with recurrent wheezing or recurrent LRTIs. These changes may be permanent, static or progressive, and may occur independently from other causes of airway obstruction such as asthma. It is therefore necessary to collect data from this early period of lung development to describe post-RSV LRTI sequelae. From a public health point of view, families and physicians could be informed of the potential sequelae after an individual RSV LRTI and the potential for improvement with time, therefore reducing the number of children incorrectly diagnosed with asthma. Furthermore, avoidance of environmental tobacco smoke and indoor biomass fuels, compliance with vaccination programs, and aggressive management of future LRTI, could be part of the management of children who previously had an RSV LRTI in infancy. These lung protective strategies are particularly relevant in LMICs which has a higher prevalence of LRTI, tuberculosis and HIV.

In infancy and early childhood, tests that require forced expiratory maneuvers, such as spirometry, are not possible, as they require understanding and cooperation from the child (162). Furthermore, spirometry only measures the pulmonary function parameters of flow and volume, thereby not providing any information on multiple other parameters and not describing the full spectrum of abnormalities that may be present post-RSV LRTI, such as resistance, reactance, and ventilation inhomogeneity (161). Therefore, using multiple different pulmonary function techniques, such as FOT, TBFVL, and MBW, the sequelae caused by RSV LRTI can be more

extensively evaluated in infancy and early childhood. Some of these measurements have not been described in children post-RSV LRTI.

Describing the lung sequelae after RSV LRTI during infancy and the time-dependent changes that may occur, requires early and regular testing with appropriate pulmonary function techniques in well-designed population studies. The candidate therefore, in fulfilment of a Doctor of Philosophy in Clinical Medicine (PhD) degree, aimed to describe the clinical and pulmonary function sequelae in black African children following severe RSV LRTI in infancy in a low-middle income setting. The evaluation of pulmonary sequelae was determined using parental based questionnaires and infant pulmonary function testing techniques which included forced oscillation technique, multiple-breath washout technique and tidal-breathing flow-volume loops at one- and two-years of age. The study also aimed to describe normative pulmonary function data for the same age groups, based upon the data acquired from the healthy controls enrolled in this study.

CHAPTER 2

2.0 Methods

2.1 Objectives

2.1.1 To determine the risk factors associated with developing pulmonary sequelae in African children at 12±2 and 24±2 months of age following severe RSV LRTI in infancy by administering a questionnaire detailing family, environmental and medical risk factors

2.1.2 To describe the clinical pulmonary sequelae in African children at 12±2 and 24±2 months of age following severe RSV LRTI in infancy through administration of a modified International Study of Asthma and Allergies questionnaire detailing the presence of past or current respiratory symptoms

2.1.3 To describe the pulmonary function sequelae in African children at 12±2 and 24±2 months of age following severe RSV LRTI in infancy through measurements of:

- Forced oscillation technique
- Tidal breath flow-volume loops
- Multiple breath inert gas washout technique

2.1.4 To describe pulmonary function parameters in healthy African children at 12±2 and 24±2 months of age through measurements of:

- Forced oscillation technique
- Tidal breath flow-volume loops
- Multiple breath inert gas washout technique

2.2 Study Design and Population

A case-control study to evaluate the clinical and pulmonary function sequelae of African children hospitalized with RSV LRTI during infancy at Chris Hani Baragwanath Academic Hospital (CHBAH) from 01 April 2016 to 31 December 2019 was undertaken.

CHBAH is a tertiary-level academic hospital in Soweto, Johannesburg, Gauteng province, South Africa. Gauteng is the most populous province of South Africa with 13.4 million inhabitants (24% of the national population), including 3.3 million children (25% of the population) under 14 years of age and 1.2 million (9% of the population) under five years of age (202). Gauteng is also the fastest growing province in the country with a 2% annual population increase. Urban and national migration, including immigration from other African countries is a large contributor to this growth (202).

Although South Africa is classified by the World Bank as an upper middle-income country, there are huge disparities between the upper and lower social quintiles. The estimated average annual household income is under R30 000; $2 100 (Exchange rate: 08 September 2021) and the unemployment rate is about 40%. In Soweto, a peri-urban mainly black-African township with an estimated population of 1.3 million, the average monthly household income is substantially lower (R5 250; $370 (exchange rate 08 September 2021)). Approximately, 40% of Soweto's population survive on under R22; $1.50 (exchange rate: 08 September 2021) a day (Stats SA definition of moderate poverty) (203). Basic electricity, water and sanitation is however available to over 90% of residents (202).

The national U5MR in South Africa has declined from 48/1000 live births in 2011 to 34.5/1000 in 2020 (5, 202, 203). The national U5MR amongst black African children however has been considerably higher at 52.4/1000 live births in 2015 (4, 202, 203).

CHBAH is the referral hospital for the communities of Soweto and surrounding areas (Region D and G of the City of Johannesburg). The only other public hospital in this setting is the primary-level Bheki Mlangeni District Hospital with a paediatric bed capacity of 34 beds (including neonatal and kangaroo mother's unit beds). In addition to being the referral centre for regions D and G, CHBAH also serves as an official tertiary-referral centre for the entire North West Province of South Africa. Approximately 25 000 children presented to or were referred to the

Ambulatory and Emergency department at CHBAH annually during the study period from 2015-2018. (Personal correspondence Dr P Vallabh – Head of Ambulatory and Emergency, Chris Hani Baragwanath Academic Hospital); 16.8% were neonates (0-28 days of age), 21.3% infants from one to under 12 months of age and 38.8% children between one and five years of age. Approximately 13.2% of these hospital visits were for LRTIs. An estimated 9 000 hospital admissions occurred annually from 2015-2018; 26.5% were neonates, 34.5% infants between one to under 12 months of age, and 31.2% children between one and five years of age. Approximately 30.1% of all admissions were for LRTIs.

The prevalence of HIV in the children in Gauteng Province, South Africa is approximately 3-4%; with the maternal HIV prevalence plateauing at ~30% over the past decade. Mother to child transmission rates of HIV are currently reported as less than 1% (204). Children infected with HIV are started on anti-retroviral therapy regardless of immunological and clinical staging.

2.2.1 Standard of care for management of LRTI at the CHBAH

Children were hospitalized and managed for acute LRTI based on a modified WHO case definition for pneumonia and severe pneumonia (205). We used the case definition LRTI instead of only pneumonia to ensure the inclusion of bronchiolitis cases. Acute LTRI was defined as acute onset cough or difficulty in breathing, with fast breathing for age, whereas severe LRTI was defined as acute onset of cough or difficulty in breathing with lower chest wall indrawing with or without fast breathing. Fast breathing in a child is defined as a respiratory rate of >50 breaths per minute in the 2-12 month age group and >40 breaths per minute in those children older than 12 months.

Children with acute LRTI or severe LRTI were admitted into one of five paediatric wards; including one that is primarily for short stay admissions (<72 hours). The standard of care during the study period was for supplemental oxygen to be administered if oxygen saturation in room air is <90%, or the child has clinical signs of severe respiratory distress. Furthermore, other supportive treatment is administered as required. In children with bronchiolitis, $beta_2$-agonists, corticosteroids and antibiotics are not routinely administered; whereas, in children with pneumonia, antibiotics are routinely administered (oral amoxicillin 30mg/kg/dose three times a

day or intravenous ampicillin 50mg/kg/dose every six hours). Children with severe disease may be monitored in the high care area, which has eight paediatric beds and three ventilators. Ventilated children may be transferred to the paediatric intensive care unit (PICU) subject to bed availability. The PICU had nine beds managed by paediatric intensivists with a nurse-to-patient ratio of 1:1.

2.2.2 Inclusion and exclusion criteria:

2.2.2.1 Inclusion Criteria

- Term infants hospitalized with severe and very severe RSV LRTI

2.2.2.2 Exclusion criteria

- Infants with a birth weight of less than 2.5kg
- Infants with any underlying congenital (ex. congenital cardiac disease, hydrocephalus), genetic (ex. trisomy 21) or medical diagnosis (ex. neurological disability, such as neuromuscular disease or cerebral palsy, hepatic abnormalities such as biliary atresia, and musculoskeletal disorders such as osteogenesis imperfecta) that may affect respiratory function
- A lower respiratory tract infection in the 4 weeks preceding the pulmonary function testing

2.3 Sample size calculation

Based on a 1:1 case-control ratio, we estimated that a sample size of 567 cases and 567 controls at the one-year visit would be required to detect a 20% difference in lung resistance between cases and controls with an 80% power, assuming a standard deviation of 1.2; and a sample size of 100 cases and 100 controls would be required to detect a 40% difference in reactance between cases and controls with an 80% power, assuming a standard deviation of 1.2.

It was estimated that two consecutive RSV seasons (2016 and 2017) would be required to reach the resistance measurement targeted sample size. These numbers were not achieved over the two-year period and the study was extended to a portion of a third RSV season (2018) – a power calculation based on the enrolled number of participants was undertaken (Table 2.1). The enrolled sample size at one year of age testing was insufficiently powered (53% power) to detect a 20% difference in resistance between cases and controls, (sufficiently powered to detect a 19.9% difference), but sufficiently powered to detect a 40% difference between cases and controls for reactance (98% power).

Table 2.1: Sample size and power calculations

Number of cases	**Power to detect 20% difference in resistance***	**Power to detect 40% difference in reactance****
100	21.5%	81.9%
200	38.3%	98.3%
300	53.1%	99.9%
400	65.3%	
500	74.9%	
600	82.2%	
700	87.6%	

* based on a two-sample t test power calculation; delta: 0.2, standard deviation: 1.2, significance level: 0.05
** based on a two-sample t test power calculation; delta: 0.49, standard deviation: 1.2, significance level: 0.05

2.4 Study method

2.4.1 Identification of eligible cases

Two surveillance studies (undertaken by the Respiratory and Meningeal Pathogens Research Unit (RMPRU) at CHBAH from December 2014) of hospitalized children were used to identify RSV LRTI cases for eligibility into our study: Surveillance of pathogen-specific causes of pneumonia and diarrhoea hospitalization in children (HREC: 131109) and Surveillance of Severe Childhood Illness in Soweto, South Africa / Babies of Soweto study (HREC: 140904). Cases were defined

as term infants hospitalised for severe or very severe RSV LRTI. Research nursing staff reviewed admission books for the admission diagnoses of LRTI in children under five years of age admitted to one of five paediatric wards. Enrolment of LRTI cases occurred seven days a week from 8:00 am until 4:00 pm. In August 2016, ward surveillance was reduced to five days per week from Monday through to Friday; an admission over the weekend that was still present on the Monday morning was screened for possible inclusion in the study.

Caregivers were approached by research staff and written informed consent was obtained. Upon caregiver consent, the infant's demographic, clinical and laboratory data were collected on a paper based case report form (CRF). Additionally, research staff reviewed and made copies of the patient's hospital charts for auditing and quality control purposes. After enrollment, patient logs were periodically reviewed to capture any changes to the level of care, including admission to the intensive care unit. Additional laboratory data, which may not have been available at the time of enrollment, was accessed through the National Health Laboratory Service (NHLS) Trakcare® system. All patient CRFs were filled out by hand and delivered to the research unit on a daily basis for auditing. After auditing by a research clinician for accuracy and completeness, the CRFs were entered into a centralized and secure electronic database system using Microsoft Visual Studio forms application and the the Microsoft SQL server database platform housed at the RMPRU.

All children with a LRTI enrolled under the surveillance studies had a nasopharyngeal swab (NPS), nasopharyngeal aspirate (NPA) or induced sputum (IS) taken for the identification of respiratory pathogens. Specimens were collected as soon as possible after arrival to the hospital, preferably within the first 72 hours (max 168 hours) of symptom onset and acute phase of illness to obtain a sample when viral shedding was greatest. The NPS was a flocked swab with a plastic shaft (FLOQS, Copan Flock Technologies, Brescia, Italy). The technique for sample collection was as follows: the swab was placed into the patient's nostril and gently advanced in the direction of the infant's ear until it was estimated to be at the mid-turbinate point or halfway between the opening of the nostril and the ear. It was then rotated several times, gently removed and placed into a collection tube with universal transport medium (UTM). Samples were placed on ice and transported to the RMPRU laboratories for processing and analysis. If the patient was intubated, a NPA was collected. Details of the date, time and name of the sampler were entered in the patient's CRF.

At the RMPRU laboratory total nucleic acid extraction using the automated NucliSENS® easyMAG® nucleic acid extraction machine was performed. A 1-Step multiplex polymerase chain reaction (PCR) assay developed at the RMPRU, which detects RSV A, RSV B, Human metapneumovirus, Influenza A, Influenza B and *Bordetella pertussis* (using IS481), was performed on the NPS. (Details of this SOP are found in appendix 2: Multiplex PCR for the detection of human Respiratory Syncytial Virus A and B, Human metapneumovirus, Influenza A and B and Bordetella species, Version 2). A cycle threshold (Ct) value of <37 was used as a cutoff for identifying positive RSV samples.

2.4.2 Identification of eligible controls

Controls were defined as term infants with no significant medical history and who were not hospitalized with a LRTI in the first year of life. Controls were matched at a 1:1 ratio for chronological age at the time of pulmonary function testing (±2 weeks of the case), gender and race. Controls were identified through a birth cohort study of 35,000 mother-newborn dyads (V98_28OB study; HREC: 140203) from 01 April 2016 until 31 December 2016, and Surveillance of Severe Childhood Illness in Soweto, South Africa / Babies of Soweto study (HREC: 140904) 01 January until 31 March 2019. There was a slight discrepancy between the number of cases and controls included in the study, this was due to the difference in the success rate of the pulmonary function testing and the inability to absolutely confirm cases and controls, and the success of the pulmonary function testing.

The V98_28OB study was a case-control study nested within a prospective, longitudinal cohort of mothers and their infants. This study aimed to establish a sero-correlate of protection, based on maternal and newborn serotype-specific levels of group B streptococcal (GBS) anti-capsular antibody, against invasive GBS disease in newborns. The study included a total of six enrolment sites and one delivery site. The infants were evaluated at six and 12 months of age by the study team to determine if hospitalization occurred in the first year of life. A list of well, non-hospitalized controls that met inclusion criteria was generated from the V98_28OB database and matched to cases. The sample function in R version 3.5.1. was used to randomly select 100 babies as a block, who were either 10-14 or 22-26 months of age and would therefore correspond to the ages of the included cases at time of pulmonary function testing. This list was worked

through from the first to the last control listed before a further list with more possibilities would be generated. Enrolment for the V98_28OB ended on 31 December 2016 and therefore no further controls were available for inclusion into the study from V98_28OB after 31 December 2018.

After 31 December 2018 identification of community controls happened through Surveillance of Severe Childhood Illness in Soweto, South Africa / Babies of Soweto study. Daily surveillance of all births at CHBAH was performed by the RMPRU and lists generated. The details of the mother was then gathered via the hospital's Medicom system (permission was obtained). Infants previously hospitalized were not eligible for participation.

2.4.3 Enrolment and testing of eligible participants

Daily in-hospital laboratory audits were conducted by the student (Dr Verwey) to identify all infants under one year of age with PCR-confirmed RSV LRTI and severe LRTI that would be eligible for inclusion in this RSV PFT study (cases). All eligible cases were informed about the study during the acute admission, or telephonically contacted after discharge, and invited to participate in the longitudinal follow-up of the study. Cases were contacted telephonically or through well baby visits one month after discharge from hospital, at six months of age (if discharged before five months of age) and at 18 months of age (Table 2.2: Schedule of visits). This was to maximise the retention of cases. Eligible controls were contacted by study staff using the generated lists and in numerical order. Ten attempts were made to contact each caregiver. Interested participants were given a date and time for the one-year visit.

Informed consent was obtained from the parent / guardian by the study staff for enrolment into this longitudinal cohort study (Appendix 3 + 4: Consent forms for cases and controls). Consent was obtained during first presentation to the pulmonary function laboratory. Consent forms were available in English; however, translators conversant in Zulu, Xhosa, Sotho, Tswana, and Afrikaans, were available for to translate for participants who were not fully versed in English. Upon enrolment, cases and controls were assigned a unique study identification number. This number was used to identify individual cases and controls throughout the duration of the study. Cases and controls

Table 2.2: Schedule of visits for cases and controls

	Visit 1	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**
	Admission to Hospital	1 month after discharge	6 months after discharge	12 months of age (10 – 14)	18 months of age (16 – 20)	24 months of age (22 – 26)
CASES						
ICFs* signed	X	X				
Inclusion/ exclusion/ withdrawal	X	X	X	X	X	X
CRF¶	X			X		X
Telephonic Follow-up		X	X		X	
PFTs†				X		X
CONTROLS						
ICFs signed				X		
Inclusion/ exclusion/ withdrawal				X	X	X
Telephonic Follow-up					X	
CRF				X		X
PFTs				X		X

*ICFs: Informed consent forms; ¶CRF: Case report forms; †PFT: Pulmonary function test

were followed up at 12 (10-14) and 24 (22-26) months of age; at these visits, PFTs, the administering of detailed case report forms specifically asking about environmental, family and medical history (Appendix 5: Case report forms for PFT study), and a CRF based on The International Study of Asthma and Allergies in Childhood Phase Three Core Questionnaire 6-7 years (ISAAC) were administered (206). Study staff were not blinded to case or control status.

The pulmonary function laboratory is a single room situated within the paediatric outpatients department of CHBAH. At the pulmonary function laboratory, participants were weighed and their length / height measured. Weight was measured to the nearest 100g with a baby scale for the one-year-olds (Nagata BW-20 Baby Scale, Nagata Scale Co., Ltd, Taiwan) and an electronic standing scale for the two-year-olds (Casa electronic body weight scale, Stingray, Cape Town, South Africa). Length in the one-year-olds and height in the two-year-olds was measured in centimetres up to the nearest centimetre by means of a length board for length and a telescopic stadiometer for height (Seca Telescopic stadiometer 220, Seca GMBH, Hamburg, Germany).

2.5 Questionnaires

At each RSV PFT visit (one and two year) CRFs were completed by the research assistants or the student. These were (Appendix 5: Case report forms for iPFT study):

1. Form 1: Linkage (Demographic details – password protected)
    a. Infant and mother / caregiver demographics
2. Form 2: History
    a. Inclusion and exclusion criteria
    b. Medical history
3. Form 3 and Form 6: Wheeze specific questionnaire - ISAAC Questionnaire
    a. Asthma and wheezing
    b. Eczema
4. Form 4 and Form 7: General visit forms:
    a. Participant details
    b. Testing laboratory details

c. Equipment calibration details
d. Data recording details.

5. Form 5 and Form 8: Excel spreadsheet containing exported iPFT testing indices

2.6 Pulmonary function testing

Pulmonary function testing was done during natural sleep – no sedation or any other medication was given on the morning of the testing. Caretakers of infants were asked to wake the child earlier than usual on the day of testing. Normal feeding of the child could continue on the morning of the testing. Caretakers were told not to let the child sleep during transport to the pulmonary function laboratory and to keep them awake until the doctor doing the testing on the day had given them instruction to put the participant to sleep. This usually took the form of the caretaker putting the child on his / her back wrapped with a blanket. This is a traditional method of transporting and caring for children in South Africa. If the child did not manage to fall asleep, they were rebooked for a future date. Participants and caregivers were remunerated for travel and time as per local ethics regulatory guidelines. A total of three scheduled visits per participant was attempted, and if natural was not achieved, only the questionnaire data would be included for that participant.

While asleep, participants were placed upon a firm mattress and covered with a blanket to keep them warm; participants were placed in a supine position with the head mildly extended (sniffing position). All PFTs were performed by the student only. During sleep and normal tidal breathing, a face mask was applied directly over the mouth and nose of the infants. PFTs were performed in the following order:

1. FOT, which measures total respiratory system impedance
2. TBFVL, which measures the tidal breathing parameters of flow and volume and calculates multiple parameters, including time to peak tidal expiratory flow (t_{PTEF}), total expiratory time (t_E), volume at peak tidal expiratory flow (V_{PTEF}) and expired tidal volume (V_E)
3. SF_6 MBW, which measures lung clearance index (LCI) and functional residual capacity (FRC) of the lung

4. Oxygen saturation at room air via pulse oximetry (Oxypleth pulse oximeter, Novametrix Medical Systems Inc., Wallingford, USA).

Each of the above tests were subject to acceptability and repeatability criteria as per American Thoracic Society / European Respiratory Society (ATS/ERS) guidelines (161). If the participant woke during the testing procedure, attempts were made to put the participant back to sleep. If these attempts failed the participant was rescheduled for another date. Each test was repeated from the beginning on each new date.

2.6.1 Forced Oscillation Technique

A custom set-up purpose-built by Prof Zoltan Hantos at the University of Szeged, Hungary, was used (207, 208). This consisted of a loudspeaker generating a 8-48Hz, 0.1-0.2kPa amplitude pressure wave which was delivered to the child via a 20cm long 1cm diameter wave tube connected in series to an anti-bacterial filter (No. 11012, Humid-Vent Filter Pedi Clean, Teleflex Medical) and a facemask (No. 415802, Anaesthetic Mask Infant size 2, Teleflex Medical). The wave tube had two identical transducers to measure the inlet and outlet pressures of the wave tube (ICS Model 33NA002D, IC Sensors, Miltipas, CA) and a pneumotachograph to measure the flow and volume through the system. (Figure 2.2)

A bias flow of medical grade oxygen at 4L/min was continuously flushed through the system to reduce the dead space (infant mask, anti-bacterial filter and flowmeter) of the circuit. The FOT set-up was supported by software designed and manufactured by Prof Zoltan Hantos's team in his laboratory in Szeged Hungary (NDAQ24 Version 2.0, 20 September 2016). Training on technique and interpretation of data was provided to the student by Prof Zoltan Hantos and Prof Diane Gray. Ongoing support was provided through a multi-national collaborative network involving regular conference calls regarding technique, software upgrades and data management.

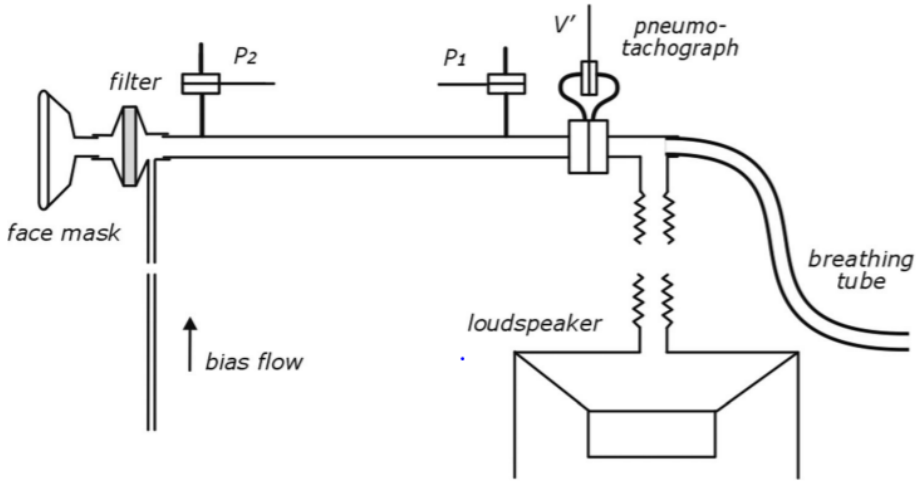


Figure 2.2: Equipment for the measurement of lung function with the forced oscillation technique (Used with permission: Prof Z Hantos)

The general application of FOT is to provide an external pressure wave via the FOT circuit to the respiratory system of the child and to define the pressure-flow relationship in terms of respiratory system impedance (Z_{rs}). Z_{rs} is the impediment to flow of the respiratory system and is made up of the respiratory system resistance (R_{rs}) and the respiratory system reactance (X_{rs}). R_{rs} is the part of the Z_{rs} that measures the frictional losses in the airways (mostly airway resistance). X_{rs} on the other hand is the component of pressure that is made up of the elastance and the inertance (I). Elastance, a measure of the elasticity in the respiratory system, is the relationship between pressure and volume and is usually reported as its reciprocal compliance, defined as the measure of the lung's ability to expand. Inertance is the relationship between pressure and volume acceleration. Resistance and elastance are calculated as follows:

Resistance (kPa/l/s) is the change in pressure (P) between two points divided by the flow ($\dot{V}$) and is calculated by the following formula:

$$\text{Resistance (R)} = (P1) - (P2) / (\dot{V})$$

Where V̇ through a tube is governed by Poiseuilles's law:

$$\Delta P = \frac{8nL\dot{V}}{\pi r4} \quad \text{or} \quad \dot{V} = \frac{\Delta P \pi r4}{8nL}$$

(n = dynamic viscosity, L = length of the tube, V̇ = volumetric flow, R = radius of the tube)

Elastance (kPa/l) is the change in pressure (P) between two points divided by the volume (V) and is usually reported as its reciprocal compliance, which is calculated by the following formula:

$$\text{Elastance} = \Delta P/\Delta V$$

$$\text{Compliance} = \Delta V/\Delta P$$

The resistance and reactance values are expressed as a function of the different oscillation frequencies produced by the FOT. During multiple-frequency pseudorandom signal (SPECTRAL) measurements, the FOT loudspeaker generates an 8-48Hz, 0.1-0.2kPa amplitude pressure wave that is delivered to the child via the wave tube, while during single sinusoidal signal (intrabreath) measurements a single wavelength (16Hz) pressure wave is delivered to the child via the wave tube. Intrabreath measurements provides additional information on the respiratory system resistance and reactance through-out an individual breath cycle, thereby providing data on both the static and dynamic components of the respiratory system.

2.6.1.1 Forced Oscillation Technique Calibration

Calibration of the FOT equipment was performed every morning according to the manufacturer's instructions and the calibration data was applied to the manufacturer's software on a daily basis. Initial calibration files for each individual FOT apparatus was devised by Prof Zoltan Hantos in his laboratory in Szeged, Hungary through multiple readings against a closed known volume, known resistance, known compliance wave tube. These initial calibration files were imbedded in the software supplied by the manufacturer and are unique for each apparatus used.

For calibration, the FOT wave tube was closed off with a stopper at the distal end of the wave tube and a narrow tube inserted into the outflow breathing tube. No bias airflow was administered. All daily calibration results were recorded in a calibration book (manual) as well as imbedded in the FOT data files measured.

2.6.1.2 Forced Oscillation Technique Procedure

After calibration had been performed, the infant mask, anti-bacterial filter and outflow tube were connected to the set-up and the bias oxygen flow was connected at 4L/min. The child's details were entered into the FOT software programme. This included the study ID number, date of birth and participant's growth parameters (height / length (cm) and weight (kg)). The child was placed in a supine position with the head tilted to a neutral / sniffing position. The mouth of the child was closed and only nasal breathing was permitted. This was to decrease the compliance of the whole respiratory system taking into consideration the increased compliance of the oral cavity represented by the cheeks and oropharynx. A tight seal was created around the mask and recordings were commenced with normal tidal breathing. SPECTRAL measurements were taken first. Recording epochs were each 60 seconds long and a minimum of three good quality SPECTRAL epochs were recorded and saved, after which a single 120 second long intrabreath epoch was recorded and saved. Real time SPECTRAL and intrabreath quality control was done visually throughout the recordings via the real-time live feed screening of the data from the recording. For each recording a segment of at least four artefact-free breaths was required.

After satisfactory SPECTRAL and intrabreath FOT measurements were completed the mask was removed from the participant's face and removed along with the bacterial filter from the wave tube. The bacterial filter would be discarded and the mask would be submerged in SaniZyme solution (SaniChem, Durban, South Africa), a four-enzyme detergent, containing lipase, cellulase, amylase and protease, for 15 minutes, after which it would be rubbed dry and allowed to air dry completely on a drying rack before further use.

2.6.1.3 Forced oscillation technique data quality control, analysis and export

The recorded participant SPECTRAL file was opened and an individual participant data file imported from the FOT participant database folder. Each recorded epoch was imported into the software programme individually and each epoch was assessed and had quality control checks administered individually per epoch. (Figure 2.3)

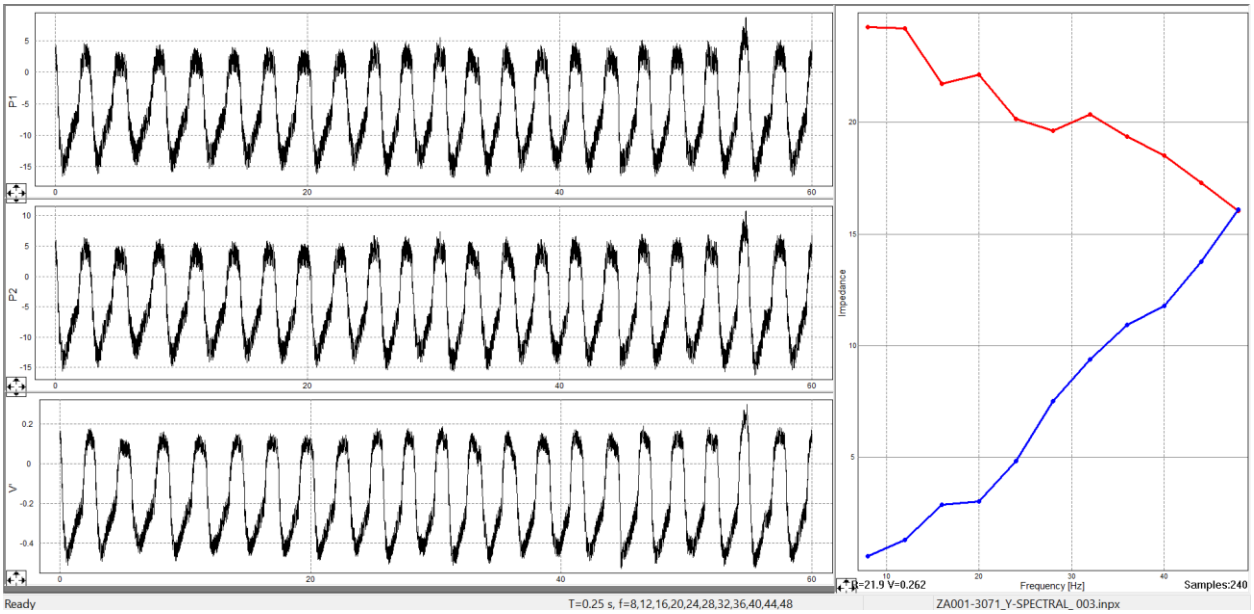


Figure 2.3: Example of an individual epoch reading recorded during SPECTRAL measurement

Once an epoch was opened, every acceptable section from each epoch was selected and added to the ensemble panel of the participant. An acceptable section of an epoch is one that starts near end-expiration of the preceding breath and continues until early inspiration of the first unacceptable breath. An acceptable section includes at least four full breaths. The breathing pattern should be regular tidal breathing as shown by regular flow signal tracing and regular pressure channel tracings.

The following breathing patterns were excluded:

1. Any air leaks in the system: this presents as a decrease in the pressure channel readings with an associated increase in oscillation amplitude in these channels. The resistance is decreased and the reactance is increased in the impedance panel.
2. Vocal cord and upper airway involvement: this has the appearance of recurrent noisy segments (higher frequency than the oscillatory signal) on the flow signal tracings. This includes any vocalisation and snoring.
3. Apnoea: this occurs when there is no breathing effort and manifests as a flat mean flow segment on the flow signal tracings.
4. Sigh: this has the appearance of stacked breaths on the flow signal tracing.
5. Glottic closure: this manifests as a flat flow signal with associated increased oscillations in the pressure channels signal tracings.

A minimum of three selections needed to be made from all the different epochs recorded per patient but not more than two selections were made from the same epoch. Each acceptable and included segment was included in the ensemble panel for the patient. (Figure 2.4)

After all SPECTRAL selections have been made the intrabreath software file was opened and the intrabreath data file of the child imported. The correct intrabreath filter selection was entered (14-16Hz) and applied. (Figure 2.5)

A good quality segment as described above for SPECTRAL measurements was selected from the intrabreath data file. The same selection criteria apply as for the SPECTRAL selections except that only one adequate intrabreath selection was required. (Figure 2.6)

The selected intrabreath segment was added to the SPECTRAL ensemble panel. If there was good concordance between the R and X at 16Hz of both the SPECTRAL and intrabreath selections the SPECTRAL data would be saved. If there was little concordance between the R and X at 16Hz of both the SPECTRAL and intrabreath selections, often due to altered mechanical status in the respiratory system between the SPECTRAL and intrabreath recordings, the test (both SPECTRAL and intrabreath) was seen is inadequate and was not included in the patient results (Failed test). (Figure 2.7)

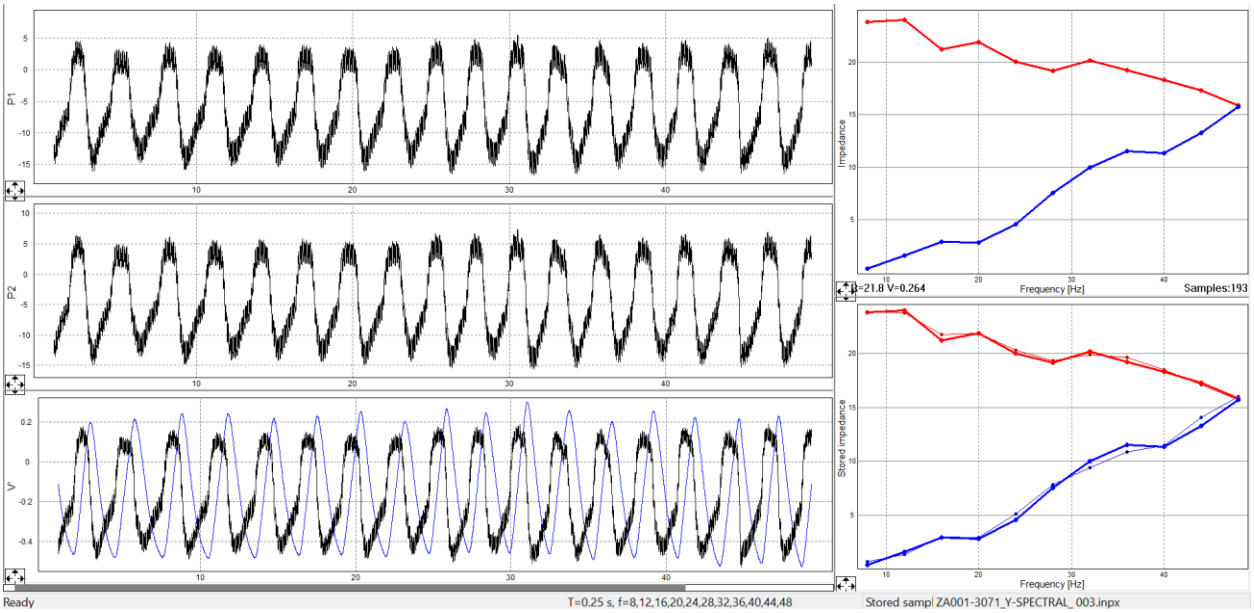


Figure 2.4: Example of a selection of an appropriate section from a SPECTRAL epoch

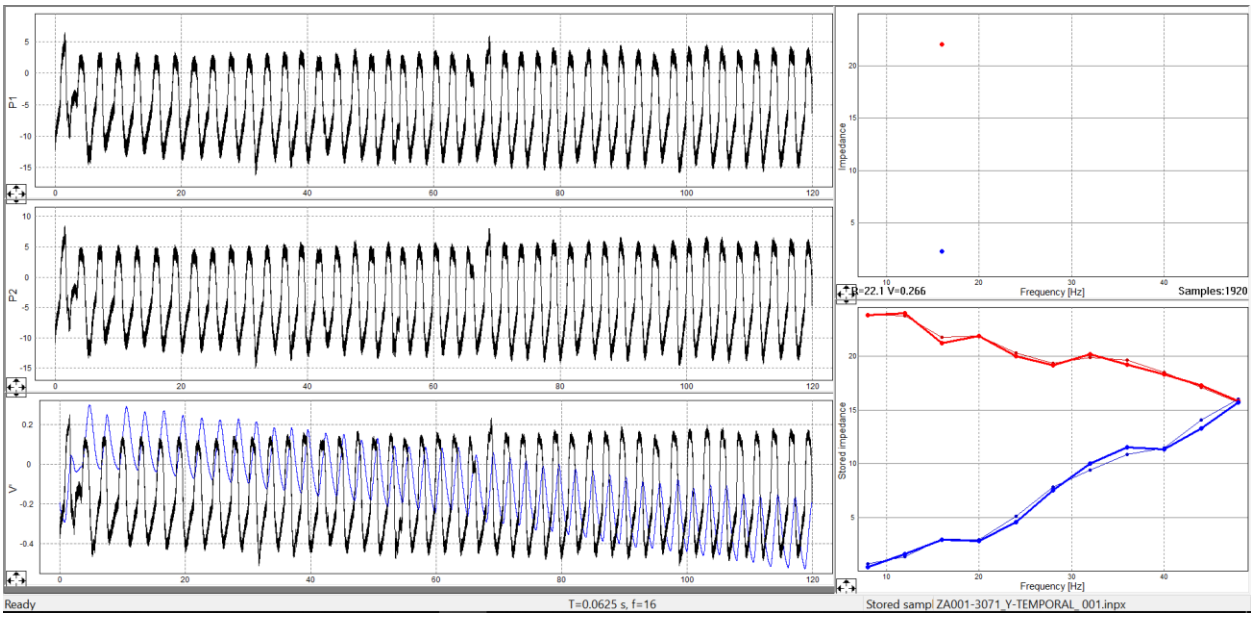


Figure 2.5: Example of an individual epoch reading recorded during intrabreath measurement

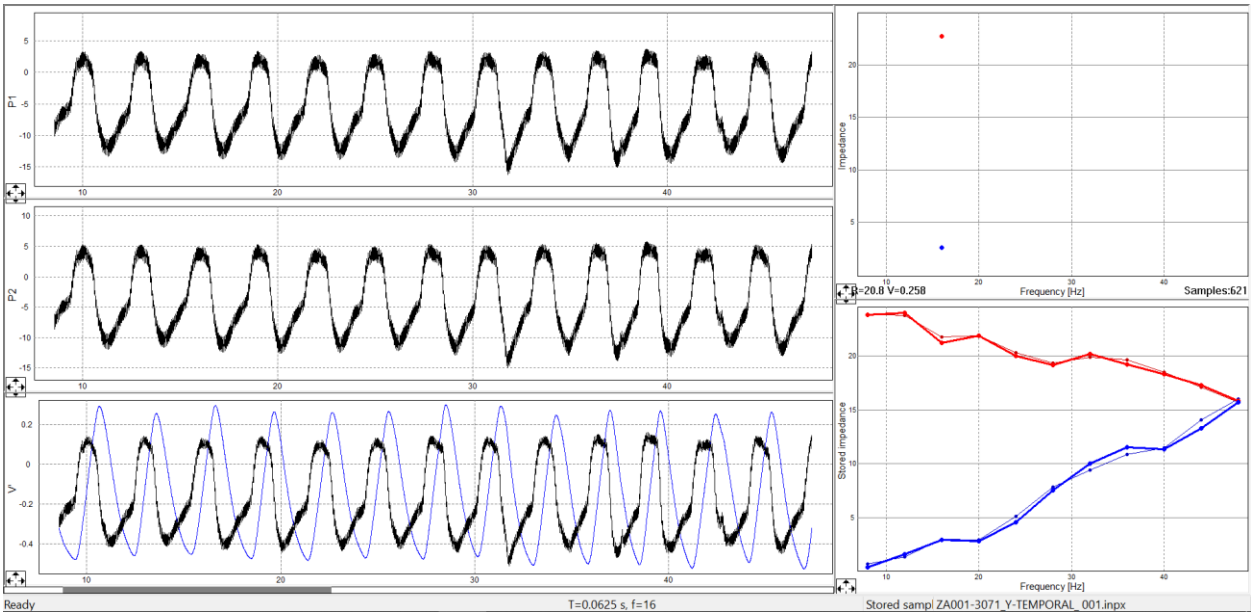


Figure 2.6: Example of a selection of an appropriate section from an intrabreath epoch

If there was concordance: the best three reproducible segments from the SPECTRAL selections were chosen to be the representative sample for analysis of the SPECTRAL results for the patient. The appropriate modelling option for the patient's age was chosen. Medium frequency ranges (20-40Hz) were chosen for the resistance calculation and all frequency ranges (8-48Hz) were chosen for the reactance (compliance and inertance) calculations. A excel spreadsheet printout of results was then supplied by the software programme for the chosen data segments. This reported the absolute values and the standard deviation of the resistance, inertance and compliance of the respiratory system. These results were then exported into the patient databases.

The intrabreath panel was accessed on the software and the impedance display options were selected. A sampling averaging time of 0.125 seconds and the impedance trend at 16Hz was chosen. The most appropriate segment following the above-mentioned criteria was then selected. The intrabreath results were then displayed in an excel spreadsheet. These results were exported into the patient databases.

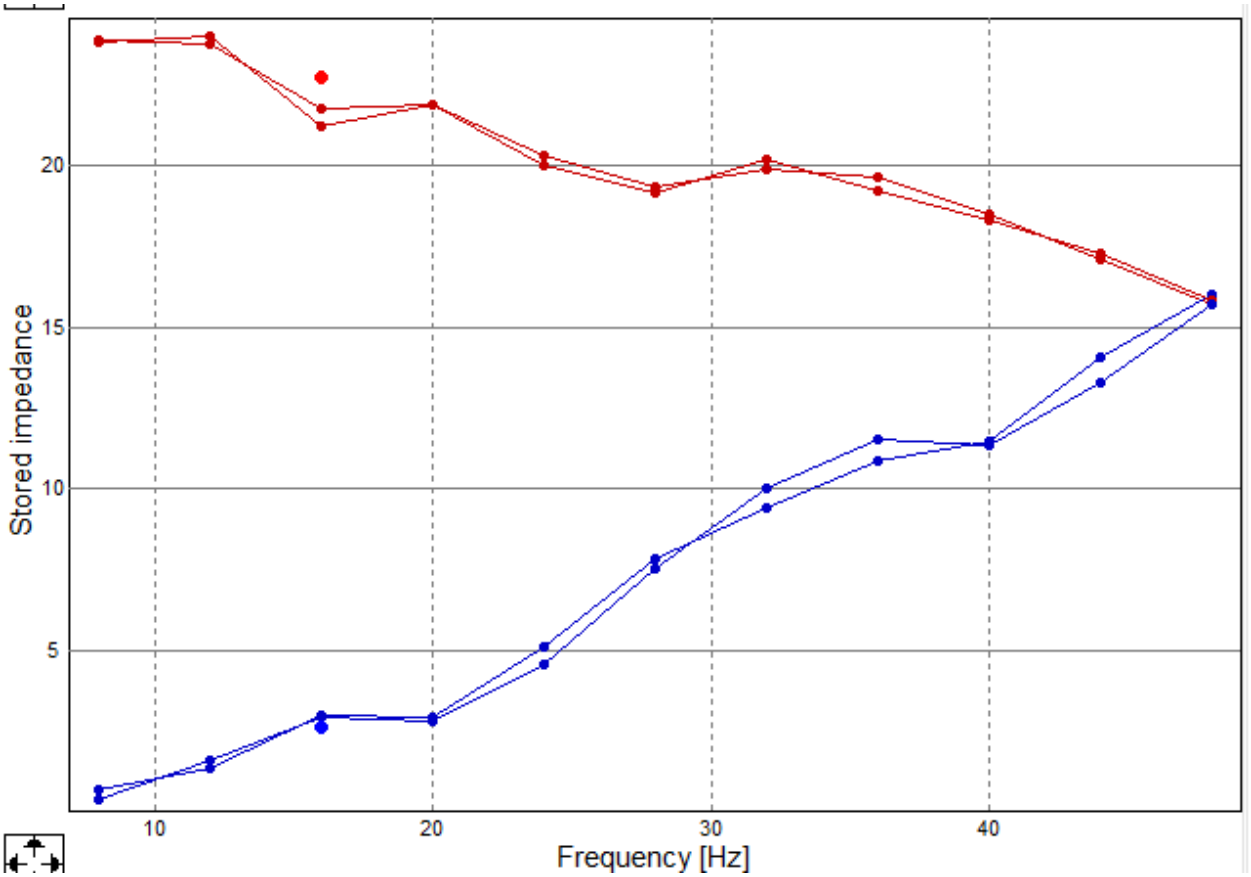


Figure 2.7: Adequate concordance between SPECTRAL and intrabreath epochs in ensemble panel

2.6.2 Tidal Breath Flow-Volume Loops and Multiple Breath Wash-out

The TBFVL and MBW lung function testing was done with the EXHALYSER® D and the accompanying SPIROWARE® 3.2.1 software package supplied by ECO MEDICS AG (Duernten, Switzerland). The EXHALYSER® D contains an ultrasonic flow measuring system for measurement of flow, volume and molecular mass; carbon dioxide (CO_2) and oxygen (O_2) measurement modules; and an inert gas FRC measurement module for infants and pre-school children. The EXHALYSER® D also contains a flow head, which is used as the interface between the patient and the EXHALYSER® D. (Figure 2.8)

a. TBFVL set-up b. MBW set-up

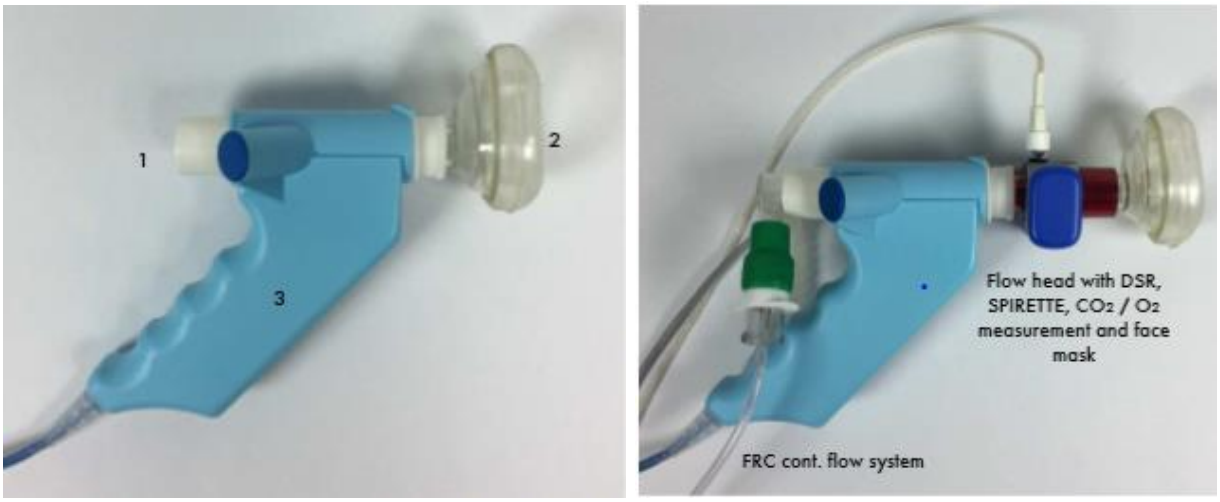


Figure 2.8: Equipment f or the measurement of lung function with the EXHALYSER D during TBFVL and MBW measurements (1. Dead space reducer, 2. Face mask, 3. Flow head) (Used with permission: Mr Dirk Wendt, CEO ECO MEDICS AG (Duernten, Switzerland)).

The flow / volume / molecular mass measurement unit consists of a lightweight flow sensor equipped with two ultrasonic transducers. These transducers transmit ultrasonic pulses in opposite directions to each other and the precise measurement of the transit time of the ultrasonic pulses determines the flow and molecular mass measurements. Sampling frequency can be up to 200Hz and is accurate within 2% between 0–50°C.

The CO_2 measurement module measures the breath-by-breath CO_2 capnography using advanced infrared absorption technology. The sensor is located in an airway adaptor and measures directly form the patient's breathing circuit. Measurement range for CO_2 is zero to 150mmHg (0-19.7% CO_2) with an accuracy of 2mmHg at 0-40mmHg, 5% at 41-70mmHg, 8% at 71-100Hg and 10% at 101-150mmHg at the sampling rate of 100Hz. The O_2 measurement module measures the breath-by-breath O_2 concentration through a sensor using laser diode absorption in the infrared spectrum. The measurement range for O_2 is 2-100% with an accuracy of 0.3% at a sampling rate of 100Hz.

The EXHALYSER® D has three predetermined measurement set-ups depending on the weight of the participants.

- Set-up 1 was used for children less than 15kg: a spirette (breathing filter) (No. M30.8042, ECOMEDICS, Switzerland or No. 2050-7, Amtronix, South Africa) was attached over a small dead space reducer (DSR) (no.M30.8001, ECOMEDICS, Switzerland). It had a tube diameter of 5mm with a resistance of <0.15cmH_2O/l/s at 0.1l/s flow and a dead space of 1.9ml. The spirette and DSR combination was inserted into the flow head of the EXHALYSER® D. Absolute alignment between these three components is required and assembly followed the instructions as per instructors manual.
- Set-up 2 was used for children between 15 and 35kg: a spirette was attached over a medium DSR (no. M30.8002, ECOMEDICS, Switzerland). It had a tube diameter of 8mm with a resistance of 0.15cmH_2O/l/s at 0.5l/s flow and a dead space of 7.2ml. The spirette and DSR combination was inserted into the flow head of the EXHALYSER® D. Absolute alignment between the three components is needed and assembly follows the instructions as per instructors manual.
- Set-up 3 is used for participants greater than 35kg and was not used during this study.

The FRC module measures the functional residual capacity of the patient's lungs. The patient breathes in a predetermined measured gas mixture of air and an inert gas. The SPIROWARE® software programme controls the titration of the gas mixtures. The FRC measurement algorithm utilizes molecular mass detection of the gasses through the ultrasonic flow measuring system to measure the FRC. During this study, 4% balanced sulphur hexafluoride (SF_6) gas mixture was used containing 4% SF_6, 21% O_2 and 75% nitrogen (Puregas, Johannesburg, South Africa).

For FRC measurement, the flow head with the DSR and spirette combination was attached to the CO_2 airway adaptor with a connected Nafion^{TM} tube and to the same disposable anaesthetic face mask described above. A low flow bypass system was connected to the rear of the flow head. This supplied a bypass flow of 200ml/s of either air or the SF_6 gas mixture to the patient. The FRC module measures the multiple breath washout indices of FRC and LCI

LCI is a measure of ventilation homogeneity within the lungs and is the number of lung volume turnovers (TO) at the first breath out of three consecutive breaths with end tidal gas concentration (C_{et}) < $1/40^{th}$ of the starting inert gas C_{et}.

LCI:

$$\frac{\text{Cumulative expiratory V} - (\text{number of breaths} * \text{post-capillary dead space ventilation})}{\text{FRC}}$$

For TBFVL measurements the flow head with the DSR and spirette combination were attached to a disposable anaesthetic face mask (No. M30.8005, ECOMEDICS, Duernten, Switzerland or No. ANCM1512, Intersurgical, Johannesburg, South Africa). TBFVL was measured during normal tidal breathing and no forced respiratory manoeuvres are required. It measured the tidal breathing parameters of flow and volume and calculated multiple parameters, including measures related to timing of peak tidal expiratory flow (time to peak tidal expiratory flow (t_{PTEF}), total expiratory time (t_E) and the ratio of t_{PTEF} and t_E (t_{PTEF}/t_E)) and measures of shape of flow-volume loop after peak flow (flow at 50% of tidal volume/peak tidal expiratory flow ($TEF_{50\%}$/PTEF)).

All measurements were done according to ATS/ERS guidelines on TBFVL and MBW except that in TBFVL instead of analysing 10 stable breaths from a recorded 30s tidal breathing measurement, we collected 100 consecutive breaths from all participants and all breaths in which the acceptability criteria of ±10% of the median tidal volume were reached were included in the analysis. A minimum of 30 acceptable breaths were required for analysis. This does conform to the section in the guidelines stating that if it is impossible to record a continuous segment of 10 stable tidal breaths, it is acceptable to combine segments from separate epochs to provide 10 breaths for analysis.

2.6.2.1 Tidal Breath Flow-Volume Loops and Multiple Breath Washout: Calibration

Daily calibration was performed on the EXHALYSER® D as per manufacturer's instructions. Firstly, the environmental settings (atmospheric temperature (°C), humidity (%) and barometric pressure (hectopascal (hPa)), measured with a mini weather station (No. WS1150, ACDC Dynamics, China) supplied by Eco Medics were entered into the software programme. Daily

early morning readings were taken during calibration and data entered into the software programme for EXHAYSER® D as well as recorded into a calibration logbook. This followed the standard BTPS (Body Temperature and Pressure Saturated) settings required for optimal reporting of lung function data as per ATS/ERS standards (161). Temperature at the flow head of the device was manually entered as 30°C with a relative humidity of 60% and patient temperature was assumed to be 37°C with a humidity of 100%. Secondly, the flow calibration for the EXHALYZER® D was performed with a 100ml test syringe (5510 series, 100ml Cal syringe, Hans Rudolph Inc., USA). Calibration was performed with 10 simulated test breaths with the calibration syringe attached to the set-up that would be used for the participant testing (DSR set-up 1 for children under 15kg or DSR set-up 2 for children >15kg). Volume offset had to be within 1% (less than 1.00ml) of the total volume over an averaged 10 breaths. Once this was achieved, the data was entered and saved in the software supplied and imbedded in the patient data files. Thirdly, a channel calibration was performed with 100% medical O_2 supplied to the EXHALYSER® D. Oxygen calibration was performed at standard atmospheric O_2 percentage (20.94%) and again at 100% supplied O_2 percentage. The acceptable range of deviation was <1.0%. Once this was achieved the data was entered and saved in the software supplied and imbedded in the patient data files. The final step of calibration was the tracer gas (SF_6) calibration. Readings of SF_6 gas concentration was made in millimols of gas. Low (28.94mmol) and high (35.22) concentrations were used for calibration. Acceptable concentrations for calibration had <1% deviation. Once this was achieved the data was entered and saved in the software supplied and imbedded in the patient data files. All the calibration data were also entered manually into the patient data files for the day.

2.6.2.2 Tidal Breath Flow-Volume Loops and Multiple Breath Wash-out: Procedure, data quality control, analysis and export

Participant details were entered into the SPIROWARE® software programme and the TBFVL software module selected. A new spirette and a clean DSR were attached to the flow head along with a clean tight fitting facemask. One hundred consecutive breaths were recorded with a

minimum of 30 non-consecutive acceptable breaths required for a test to be acceptable. If the test was deemed acceptable it is concluded and saved.

Next, the MBW measurement was commenced. The facemask was removed, the CO_2 adaptor with the Nafion^{TM} tube was connected to the flow head and the mask was reapplied to the face of the participant ensuring a tight leak free seal. The low flow bypass connection was attached to the rear of the flow head. A bias flow of 200ml/s medical air was supplied to the participant via the low flow bypass. Regular constant tidal breathing was required during the testing. Recordings were initiated as per supplied software instructions. An initial pre-test phase of regular tidal breaths was initiated. Once tidal breathing was stable, the wash-in phase of the test would commence with the supply of the tracer gas (SF_6) to the participant. This would continue until there was equilibrium of the tracer gas concentration between inspiratory and expiratory breaths (4% SF_6 concentration). Once equilibrium has been reached, a further five breaths were required before the wash-out phase would commence. In the wash-out phase, the tracer gas would be flushed out with medical air until the final concentration of the tracer gas was $1/40^{th}$ (2.5%) of the wash-in concentration. A further five breaths was provided for an acceptable end to the test. A minimum of three good quality SF_6 MBWs were recorded. All tests with uneven breathing, sighs, apnoeas, glottis closure or wakening during five pre-wash-out phase breaths or during the wash-out phase of the test were deemed unacceptable and excluded from analysis. Three acceptable FRC recordings within 10% of each other, and the LCIs within one turnover of each other was required. If three acceptable recording were not possible, two FRCs within 5% of each other and the LCIs within one turnover of each other was deemed acceptable. Both TBFVL and MBW SF_6 data from the patient was then exported to an excel spreadsheet and the required data extracted and exported to the patient study database.

2.7 Data Management

Cases and controls were assigned a unique study identification number that was used throughout the duration of the study. All identifiers were removed from the files and kept in a separate file that only the student had access to. Data was entered onto paper CRFs and then captured in duplicate into a centralized and secure electronic database system using Microsoft Visual Studio

forms application and the the Microsoft SQL server database platform housed at the RMPRU, by two data capturers employed by the RMPRU. Data entry was centralised at the RMPRU and supervised by the database manager. Any queries or missing entries were returned to study staff for correction or completion.

Data from the PFTs were generated by the NDAQ24 Version 2.0, 20 September 2016 (FOT) and SPIROWARE® (TBFVL and MBW) software programmes. These data were then exported to an excel data spreadsheet.

2.8 Data Analysis

All statistical analyses were performed by the student using Stata 13 (StataCorp (Texas, USA)). For continuous variables, the mean and standard deviation was used if the data was normally distributed and the median and interquartile range (IQR) if not. Proportions and percentages were used for categorical variables. Comparisons between cases and controls were made using the Student t-test or Mann-Whitney U test as indicated for continuous variables, and the Chi-square or Fisher's exact test for categorical variables. Odds ratios (OR) with 95% confidence intervals (95% CI) were reported. A p-value is less than 0.05 was considered statistically significant.

To determine the risk factors associated with developing pulmonary sequelae in African children at 12±2 and 24±2 months of age following severe RSV LRTI in infancy

Comparisons from the univariate analysis with a p-value of <0.2 were included in the multivariate regression analysis to determine independent risk factors associated with clinical and pulmonary function sequelae. P-values and adjusted odds ratios (95% confidence interval) were reported for logistic regression, and p-values and coefficients were reported for the linear regression analysis. An arbitrary p-value (<0.2) was chosen for the inclusion of independent variables from the univariate analysis into the multivariate regression mode. This was done to ensure that all variables that are or trended towards statistical significance are included in the multivariate analysis.

To describe the clinical pulmonary sequelae in African children at 12±2 and 24±2 months of age following severe RSV LRTI in infancy

Conditional logistic regression was used to determine the odds of developing clinical pulmonary sequelae in cases compared to controls; we controlled for confounding factors that were significant in the univariate analysis as identified from the administered questionnaire and patient data. A univariate regression analysis was initially run for all clinical outcome variables in one- and two-year-old children and all independent variables with a p-value of <0.2 were included in the multivariate regression analysis for clinical outcome variables. An arbitrary p-value (<0.2) was chosen for the inclusion of independent variables from the univariate analysis into the multivariate regression mode. This was done to ensure that all variables that are or trended towards statistical significance are included in the multivariate analysis.

To describe the pulmonary function sequelae in African children at 12±2 and 24±2 months of age following severe RSV LRTI in infancy we measured pulmonary function through forced oscillation technique, tidal breath flow-volume loops, and multiple breath inert gas washout technique.

Linear regression analysis was used for continuous outcome variables to determine associations between risk factors and the development of pulmonary function sequelae in cases compared to controls. A univariate regression analysis was initially run for all pulmonary function outcome variables in one- and two-year-old children and all independent variables (risk factors) with a p-value of <0.2 were included in the multivariate regression analysis. An arbitrary p-value (<0.2) was chosen for the inclusion of independent variables from the univariate analysis into the multivariate regression mode. This was done to ensure that all variables that are or trended towards statistical significance are included in the multivariate analysis.

To describe pulmonary function parameters in healthy African children at 12±2 and 24±2 months of age we measured pulmonary function through forced oscillation technique, tidal breath flow-volume loops, and multiple breath inert gas washout technique.

Normal pulmonary function parameters were summarised using mean and standard deviations for continuous variables with normal distribution; median, interquartile range (IQR) and range for continuous data without normal distribution. Data were reported in tables and in centile charts stratified to the height of the participants, as well as the gender. Data were displayed in tables and graphs.

2.9 Ethics

Ethics submission was approved by the University of the Witwatersrand Human Research Ethics Committee: (Medical) on 08 February 2016. Clearance certificate No: M160224. (Appendix 6-8: HREC clearance certificates). Permission to conduct research at Chris Hani Baragwanath Academic Hospital granted via the Medical Advisory Committee on 17 February 2016. (Appendix 9: MAC clearance certificate)

The following amendments were required:

1. Amendment to ethics submission for change of title approved by the University of the Witwatersrand Human Research Ethics Committee: (Medical) on 04 December 2018. Clearance certificate No: M160224.
2. Amendment to ethics submission for inclusion of community controls approved by the University of the Witwatersrand Human Research Ethics Committee: (Medical) on 04 December 2018. Clearance certificate No: M160224.
   Ethics waiver form approved by the University of the Witwatersrand Human Ethics Committee: (Medical) on 07 December 2018 for the systematic literature review: Pulmonary function sequelae after respiratory syncytial virus lower respiratory tract infection in children: a systematic review. Reference: W-CP-181207-4.

2.10 Funding

The study was funded through: (Appendix 10: Study budget)

1. Respiratory and Meningeal Pathogens Research Unit

2. SAMRC/WITS Clinician Researcher Programme: PhD scholarships in Clinical Research: 2016-2019
3. South African Thoracic Society Astra-Zeneca Educational grant

CHAPTER 3

3.0 Results

3.1 Basic demographics and clinical characteristics of children enrolled into the study

Three hundred and eight one-year-old (57% male; median age 12.4 months (IQR 11.5-13.7)) and 214 two-year-old (54% male; median age 24.63 (23.87-25.46)) children were enrolled into the RSV PFT study as cases, and 292 one-year-old (53% male; median age 12.5 months (IQR 11.7-13.0) and 209 two-year-old (46% male; median age 23.90 (23.18-24.73)) children, not previously admitted for an LRTI as controls. One-year-old cases and controls were similar with respect to median z-scores for weight-for-age (0.29 (IQR -0.46-1.0) vs (0.18 (IQR -0.59-0.96)), height-for-age (-0.53 (IQR -1.31-0.41) vs -0.62 (IQR -1.39-0.453)), and body mass index (0.78 (IQR -0.09-1.62) vs (0.64 (IQR -0.23-1.57)). Similarly, two-year-old cases were similar in weight-for-age z-scores (-0.50 (IQR -0.74-0.78) vs -0.08 (IQR -0.79-0.52)), height-for-age (-0.15 (IQR -0.78-0.66) vs -0.09 (IQR -1.16-0.76)), and BMI (0.15 (IQR -0.74-0.95) vs -0.7 (IQR -0.87-1.11)).

Their basic demographics are described in Table 3.1 and 3.2. One-year-old cases compared well to controls, but two-year-old cases were significantly older than controls and weighed more.

Risk factors for developing respiratory disease are described in Tables 3.3 and 3.4. One and two-year-old cases and controls were similar with regards to mother's HIV status, maternal tobacco smoking during pregnancy, environmental tobacco smoke exposure, modality of infant feeding, and socioeconomic indicators, however, one and two-year-old cases were more likely to have attended crèche, and two-year-old cases to have pets in the household. Only six cases from either time-points required invasive ventilation in the intensive care unit.

Table 3.1: Demographic characteristics of one-year-old cases and controls

Variable	**Cases (n*=308)**	**Controls (n=292)**
Age (Months) (Median (IQR†), Range)	12.41 (11.48-13.66), 8.33-17.1	12.48 (11.72-13.07), 10.29-16.52
Gender Male (n/%)	174 (56.5)	155 (53.1)
Black African Race (n/%)	308 (100)	292 (100)
Weight (kg) (Median (IQR), Range)	9.9 (9.0-10.6), 5.4-14.8	9.8 (8.8-10.5), 6.7-15.1
Height (cm) (Median (IQR), Range)	74 (72-77), 54-89	74 (72-77), 62-88
Birth weight (g) (Median (IQR), Range)	3095 (2840-3400), 2500-4600	3138 (2900-3380), 2495-4875
HIV status (n/%) - Unexposed - Exposed uninfected - Infected - Unknown	 229 (74.4) 67 (21.7) 11 (3.6) 1 (0.3)	 214 (73.3) 74 (25.3) 4 (1.3) 0 (0.0)
Underlying medical conditions¶ (n/%)	0 (0.0)	0 (0.0)
Age (Months) at RSV§ admission (Median (IQR), Range)	4.48 (2.26-6.85), 0.16-12	N/A
Time (months) between RSV admission and 1 year visit (Median (IQR), Range)	7.60 (4.77-10.68), 0.67-14.55	N/A
Duration of RSV admission (days) (Median (IQR), Range)	2 (1-4), 1-23	N/A
Required oxygen during RSV admission (n/%)	103 (34.7)	N/A

*n: number; †IQR: Interquartile range; ¶Underlying medical conditions: congenital (ex. congenital cardiac disease, hydrocephalus), genetic (ex. trisomy 21) or medical diagnosis (ex. neurological disability, such as neuromuscular disease or cerebral palsy, hepatic abnormalities such as biliary atresia, and musculoskeletal disorders such as osteogenesis imperfecta) that may affect respiratory function; §RSV: respiratory syncytial virus

Table 3.2: Demographic characteristics of two-year-old cases and controls

Variable	**Cases (n*=214)**	**Controls (n=209)**
Age (months) (Median (IQR†), Range)	24.63 (23.87-25.46), 21.14-28.61	23.90 (23.18-24.73), 18.03-26.9§
Gender Male (n/%)	116 (54.2)	95 (45.5)
Race Black African (n/%)	214 (100.0)	209 (100.0)
Weight (kg) (Median (IQR), Range)	12.0 (10.9-13.3), 9-20.4	11.6 (10.6-12.6), 7.5-20.1¶
Height (cm) (Median (IQR), Range)	87 (85-90), 70-97	86 (83-89), 72-100
Birth weight (g) (Median (IQR), Range)	3102 (2895-3400), 2500-4600	3175 (2855-3445), 2545-4540
HIV status (n/%) - Unexposed - Exposed uninfected - Infected - Unknown	 157 (73.4) 45 (21.0) 3 (1.4) 9 (4.2)	 150 (71.8) 52 (24.9) 1 (0.5) 6 (2.9)
Underlying medical conditions‡ (n/%)	0 (0.0)	0 (0.0)
Age (Months) at RSV⁑ admission (Median (IQR), Range	4.48 (2.26-6.85), 0.16-12	N/A
Time (months) between RSV admission and 2 year visit (Median (IQR), Range)	20.82 (1651-22.79), 12.4-26.90	N/A
Duration of RSV admission (days)(Median (IQR), Range)	2 (1-5), 1-23	N/A
Required oxygen during RSV admission (n/%)	69 (34.9%)	N/A

*n: number; †IQR: interquartile range; §p<0.001; ¶p=0.007; ‡Underlying medical conditions: congenital (ex. congenital cardiac disease, hydrocephalus), genetic (ex. trisomy 21) or medical diagnosis (ex. neurological disability, such as neuromuscular disease or cerebral palsy, hepatic abnormalities such as biliary atresia, and musculoskeletal disorders such as osteogenesis imperfecta) that may affect respiratory function; ⁑RSV: respiratory syncytial virus.

Table 3.3: Risk factors for respiratory disease in one-year-old cases and controls

Variable	Cases (n*=308)	Controls (n=292)	p-value	OR† (95% CI¶)
Infant factors				
Neonatal admission post-delivery (n/%)	47 (15.3)	24 (8.2)	0.008	2.00 (1.16-3.51)
Duration of admission post-delivery (days) (Median (IQR§), Range)	3 (2-7), 1-30	3 (2-7), 1-25	0.587	
Required oxygen post-delivery (n/%)	28 (9.1)	10 (3.4)	0.006	2.72 (1.25-6.39)
Duration of oxygen post-delivery (days) (Median (IQR), Range)	3 (1-10), 1-18	2 (1-4), 1-5	0.295	
Hospitalized prior to RSV admission (n/%)	57 (18.5)	41 (14.0)	0.139	1.39 (0.88-2.21)
Weight loss in last 6 months (n/%)‡	67 (21.8)	43 (14.7)	0.352	1.57 (1.01-2.46)
TB contact (n/%)	14 (4.5)	17 (5.9)	0.436	0.75 (0.34-1.65)
Type of feeding during first 4 months (n/%)			0.867	
-Breastfeeding	199 (64.6)	187 (64.0)		
-Formula	70 (22.7)	61 (20.9)		
-Mixed feeding	39 (12.7)	44 (15.1)		
Maternal factors				
Mother's HIV status: Positive (n/%)	71 (24.1)	85 (29.6)	0.131	0.75 (0.51-1.11)
Smoke exposure from primary caregiver during pregnancy (n/%)‡	12 (3.9)	8 (2.7)	0.449	1.42 (0.52-4.06)
Does primary caregiver consume alcohol? (n/%)‡	39 (12.7)	60 (20.6)	0.008	0.56 (0.35-0.88)
Alcohol consumption by primary caregiver during pregnancy (n/%)‡	10 (3.3)	7 (2.4)	0.549	1.35 (0.46-4.23)
Maternal diagnosed asthma (n/%)‡	11 (3.6)	7 (2.4)	0.388	1.52 (0.53-4.70)
Paternal factors				
Paternal diagnosed asthma (n/%)‡	8 (2.6)	8 (2.7)	0.876	0.92 (0.30-2.87)
Sibling factors				
Sibling diagnosed asthma (n/%)‡	10 (3.2)	2 (0.6)	0.024	4.92 (1.03-46.41)
Environmental factors				
Smoke exposure within home (n/%)‡	48 (15.6)	58 (19.9)	0.169	0.74 (0.48-1.16)
Home construction (Brick) (n/%)	252 (81.8)	223 (76.4)	0.101	1.39 (0.92-2.11)
Total rooms in the house (Median (IQR), Range)	4 (2-5), 1-11	4 (1-4), 1-16	0.121	

Table 3.3 cont.: Risk factors for respiratory disease in one-year-old cases and controls

Variable	**Cases (n=308)**	**Controls (n=292)**	**p-value**	**OR (95% CI)**
Main energy source (Electricity) (n/%)	297 (96.4)	280 (95.9)	0.732	1.16 (0.46-2.95)
Main water source of household (Indoor) (n/%)	176 (57.1)	154 (52.7)	0.279	1.19 (0.85-1.67)
Type of toilet (Flush) (n/%)	288 (93.5)	265 (90.8)	0.209	1.47 (0.77-2.83)
How many people sleep in the same room as the child (Mean (SD°), Range)	2.28 (0.91), 1-6	2.05 (0.95), 1-9	0.003	
Total number of people living in the house (Mean (SD), Range)	5.51 (2.63), 2-15	5.17 (2.35), 2-19	0.090	
Under-5s attending crèche in the household (Mean (SD), Range)	0.52 (0.77), 0-6	0.31 (0.58), 0-3	<0.001	
Child attends crèche (n/%)	80 (26.0)	36 (12.3)	<0.001	2.50 (1.59-3.96)
Pets in the household (n/%)	57 (18.5)	45 (15.4)	0.313	1.25 (0.79-1.96)
Exposure to farm animals (n/%)	7 (2.3)	6 (2.1)	0.798	1.15 (0.33-4.21)
RTHC‡‡ available (n/%)	297 (96.4)	278 (95.2)	0.454	1.36 (0.56-3.37)

*n: number; †OR: Odds ratio; ¶CI: Confidence interval; §IQR: Interquartile range; ‡Parental reporting; °SD: Standard deviation;
‡‡RTHC: Road to Health card

Table 3.4: Risk factors for respiratory disease in two-year-old cases and controls

Variable	Cases (n*=214)	Controls (n=209)	p-value	OR† (95% CI¶)
Infant factors				
Neonatal admission post-delivery (n/%)	31 (14.5)	32 (15.3)	0.812	1.067 (0.60-1.89)
Duration of admission post-delivery (days) (Median (IQR§), Range)	3 (2-7), 1-21	5 (2-8), 1-31	0.622	
Required oxygen post-delivery (n/%)	18 (8.4)	16 (7.7)	0.792	1.10 (0.51-2.37)
Duration of oxygen post-delivery (days) Median (IQR), Range)	2 (1-7), 0-14	6 (2-7), 1-16	0.241	
Weight loss in last 6 months (n/%)‡	38 (17.8)	52 (24.9)	0.008	0.54 (0.33-0.87)
TB contact (n/%)	10 (4.7)	1 (0.5)	0.008	9.85 (1.37-429.60)
Type of feeding during first 4 months (n/%) -Breastfeeding -Formula -Mixed feeding	 136 (63.6) 51 (23.8) 27 (12.6)	 115 (55.0) 56 (26.8) 38 (18.2)	0.150	
Maternal factors				
Mother's HIV status: Positive (n/%)	48 (23.6)	58 (29.6)	0.179	0.74 (0.46-1.18)
Smoke exposure from primary caregiver during pregnancy (n/%)‡	6 (2.8)	8 (3.8)	0.550	0.72 (0.20-2.42)
Does primary caregiver consume alcohol (n/%)‡	31 (14.5)	35 (16.8)	0.494	0.83 (0.47-1.46)
Alcohol consumption by primary caregiver during pregnancy (n/%)‡	7 (3.3)	8 (3.8)	0.749	0.85 (0.26-2.72)
Maternal diagnosed asthma (n/%)‡	7 (3.3)	8 (3.8)	0.859	1.09 (0.37-3.32)
Paternal factors				
Paternal diagnosed asthma (n/%)‡	6 (2.8)	3 (1.4)	0.382	1.85 (0.39-11.58)
Sibling factors				
Sibling diagnosed asthma (n/%)‡	8 (5.8)	8 (6.3)	0.788	0.87 (0.28-2.76)
Environmental factors				
Smoke exposure within home (n/%)‡	42 (19.6)	39 (18.7)	0.801	1.06 (0.64-1.78)
Home construction (Brick) (n/%)	178 (83.2)	162 (77.5)	0.142	1.43 (0.86-2.40)
Total rooms in the house (Median (IQR), Range)	4 (2-4), 1-11	3 (1-4), 1-9	0.076	
Main energy source (Electricity) (n/%)	207 (96.7)	198 (94.7)	0.310	1.64 (0.57-5.10)

Table 3.4 cont.: Risk factors for respiratory disease in one-year-old cases and controls

Variable	**Cases (n=214)**	**Controls (n=209)**	**p-value**	**OR (95% CI)**
Main water source of household (Indoor) (n/%)	124 (57.9)	103 (49.3)	0.074	1.42 (0.95-2.12)
Type of toilet (Flush) (n/%)	202 (94.4)	191 (91.4)	0.229	1.59 (0.70-3.71)
How many people sleep in the same room as the child (Mean (SD°), Range)	2.33 (0.91), 1-5	2.17 (0.86), 1-6	0.067	
Total number of people living in the house (Mean (SD), Range)	5.52 (2.36), 2-16	5.09 (2.17), 2-14	0.054	
Under-5s attending crèche in the household (Mean (SD), Range)	0.50 (0.67), 0-5	0.38 (0.56), 0-2	0.057	
Child attends crèche (n/%)	61 (28.5)	79 (37.8)	0.042	0.66 (0.43-1.01)
Pets in the household (n/%)	47 (22.0)	26 (12.4)	0.010	1.98 (1.14-3.49)
Exposure to farm animals (n/%)	6 (2.8)	5 (2.4)	0.710	1.26 (0.31-5.29)
RTHC‡‡ available (n/%)	208 (97.2)	198 (94.7)	0.198	1.93 (0.64-6.46)

*n: number; †OR: Odds ratio; ¶CI: Confidence interval; §IQR: Interquartile range; ‡Parental reporting; °SD: Standard deviation;
‡‡RTHC: Road to Health card

3.2 Modified ISAAC questionnaire undertaken for children enrolled in the study

The responses to the questions of the modified ISAAC questionnaire detailing the presence of wheeze, admission for wheezing, admission for chest infections and other indicators of respiratory health for one and two-year-old children are detailed in Table 3.5 and 3.6.

Following the admission for RSV-LRTI, one-year-old cases were more likely to have experienced wheezing or whistling in the chest (OR 3.98 (95% CI 2.76-5.75)), had any admission for wheezing or whistling in the chest (OR 7.69 (95% CI 4.33-14.39)) or for a chest infection (OR 14.81 (95% CI 6.31-42.26)), had reported sleep that was disturbed due to wheezing or whistling in the chest (OR 7.6 (95% CI 4.94-11.73)), had a dry cough at night, apart from when he/she has a cold or an infection (OR 4.51 (3.14-6.49)), and to have received treatment for wheezing or whistling in the chest (OR 2.55 (95% CI 1.47-4.53)) than controls. In cases the time period being assessed was that between the RSV-LRTI admission and the one year visit, while in controls it included the full first year of life.

These findings were similar in two-year-old children, where during the past 12 months, cases were more likely to have experienced wheezing or whistling in the chest (OR 8.43 (95% CI 5.20-13.77)), experienced wheezing or whistling in the chest during activity (OR 14.61 (95% CI 7.52-30.40)), have received treatment for wheezing or whistling in the chest (OR 20.89 (95% CI 10.69-43.56)), had any admission for wheezing or whistling in the chest (OR 91.04 (95% CI 23.56-769.85)) or for any chest infection (OR 33.60 (95% CI 10.63-169.11)), had reported sleep that was disturbed due to wheezing or whistling in the chest (OR 22.56 (95% CI 12.70-40.97)), had a dry cough at night, apart from when he/she has a cold or an infection (OR 8.38 (95% CI 5.24-13.44)), or had been diagnosed with asthma by a doctor (OR 12.75 (95% CI 1.85-547.39)).

Table 3.5: Modified ISAAC* questionnaire administered for one-year-old children

Variable	**Cases (n†=308)**	**Controls (n=292)**	**OR¶ (95% CI§)**
Did your child experience any wheezing or whistling in the chest before the RSV‡ admission (n/%)	236 (76.6)	N/A	
Has your child experienced any wheezing or whistling in the chest over the past year‡ (n/%)	170 (55.2)	69 (23.6)	3.98 (2.76-5.75)
If yes, number of episodes per year (n/%)			
1-3	115 (37.3)	50 (17.1)	2.88 (1.94-4.32)
>3	55 (17.9)	19 (6.5)	3.12 (1.76-5.72)
Has your child had any admissions for wheezing or whistling in the chest in the past year‡ (n/%)	95 (30.8)	16 (5.5)	7.69 (4.33-14.39)
If yes, number of episodes per year (n/%)			
1-2	64 (20.8)	15 (5.1)	4.84 (2.64-9.38)
>2	31 (10.1)	1 (0.3)	33.35 (5.46-1363.42)
Has your child had any admissions for a chest infection in the past year‡ (n/%)	73 (23.7)	6 (2.1)	14.81 (6.31-42.26)
If yes, number of episodes per year (n/%)			
1-2	49 (15.9)	6 (2.1)	9.02 (3.76-26.12)
>2	24 (7.8)	0 (0.0)	24.59 (3.94-1014.49)
Has your child's sleep ever been disturbed due to wheezing or whistling in the chest in the past year‡ (n/%)	161 (52.3)	37 (12.7)	7.6 (4.94-11.73)
If yes, number of times per week during last year (n/%)			
1-2	50 (16.2)	15 (4.9)	3.58 (1.92-7.02)
>2	111 (36.0)	22 (7.6)	6.92 (4.16-11.87)
If yes, number of times per week during last year (Mean (SD°), Range)	3.0 (1.1), 1-4	2.8 (1.1), 1-4	
Has your child ever had a dry cough at night, apart from when he/she has a cold or an infection, during the past year‡ (n/%)	189 (61.3)	76 (26.0)	4.51 (3.14-6.49)

Table 3.5 cont.: Modified ISAAC questionnaire administered for one-year-old children

Variable	**Cases (n=308)**	**Controls (n=292)**	**OR (95% CI)**
If yes, number of nights per week, during last year (n/%) 1-2 >2	 53 (17.2) 136 (44.2)	 38 (13.0) 37 (12.7)	 1.39 (0.86-2.25) 5.45 (3.55-8.46)
If yes, number of nights per week during last year (Mean (SD), Range)	3.09 (0.99), 1-4	2.55 (1.13), 1-4	
Has your child ever had wheezing or whistling in the chest during activity in the past year (n/%)	107 (34.7)	15 (5.1)	9.83 (5.48-18.67)
Has your child been diagnosed with asthma by a doctor in the past year (n/%)	14 (4.6)	2 (0.7)	6.90 (1.56-62.97)
Has your child received treatment for wheezing or whistling in the chest in the past year (n/%)	53 (17.2)	22 (7.5)	2.55 (1.47-4.53)
Has your child ever been diagnosed with eczema by a doctor in the past year (n/%)	47 (15.3)	50 (17.1)	0.87 (0.55-1.38)
Has your child ever had an itchy rash that comes and goes in the past year (n/%)	106 (34.4)	108 (40.0)	0.89 (0.63-1.27)
Has your child ever had an itchy rash that affects the folds of the elbows, behind the knees, front of the ankles, buttocks, around the eyes during the past year (n/%)	103 (33.4)	89 (30.5)	1.15 (0.80-1.64)

*ISAAC: International Study of Asthma and Allergies in Childhood; †n: number; ¶ OR: Odds ratio; §CI: Confidence interval; ‡For cases questions apply to the remainder of the year after the RSV admission; °SD: Standard deviation

Table 3.6: Modified ISAAC* questionnaire administered for two-year-old children

Variable	**Cases (n†=214)**	**Controls (n=209)**	**OR¶ (95% CI§)**
Has your child experienced any wheezing or whistling in the chest over the past year (n/%)	131 (61.2)	34 (16.3)	8.43 (5.20-13.77)
If yes, number of episodes per year (n/%)			
1-3	87 (40.7)	26 (12.4)	6.96 (4.16-11.77)
>3	44 (20.6)	8 (3.8)	6.50 (2.92-16.37)
Has your child had any admissions for wheezing or whistling in the chest in the past year (n/%)	99 (46.3)	3 (1.4)	91.04 (23.56-769.85)
If yes, number of episodes per year (n/%)			
1-2	76 (35.5)	3 (1.4)	37.82 (12.00-189.92)
>2	23 (10.7)	0 (0.0)	25.04 (3.97-1035.97)
Has your child had any admissions for a chest infection in the past year (n/%)	69 (32.2)	3 (1.4)	33.60 (10.63-169.11)
If yes, number of episodes per year (n/%)			
1-2	44 (20.6)	2 (1.0)	26.79 (6.78-230.12)
>2	25 (11.7)	1 (0.5)	27.51 (4.39-1134.73)
Has your child's sleep ever been disturbed due to wheezing or whistling in the chest in the past year (n/%)	148 (69.2)	20 (9.6)	22.56 (12.70-40.97)
If yes, number of times per week during last year (n/%)			
1-2	52 (24.3)	13 (6.2)	6.84 (3.53-14.07)
>2	96 (44.9)	7 (3.3)	23.48 (10.42-61.54)
If yes, number of times per week during last year (Mean (SD‡), Range)	3.0 (1.0), 1-4	2.35 (1.2), 1-4	
Has your child ever had a dry cough at night, apart from when he/she has a cold or an infection, during the past year (n/%)	142 (66.4)	42 (20.1)	8.38 (5.24-13.44)
If yes, number of nights per week, during last year (n/%)			
1-2	34 (15.9)	23 (11.0)	1.53 (0.84-2.83)
>2	108 (50.5)	19 (9.1)	10.19 (5.80-18.49)
If yes, number of nights per week during last year (Mean (SD), Range)	3 (3-4), 1-4	2 (2-4), 1-4	
Has your child ever had wheezing or whistling in the chest during activity in the past year (n/%)	97 (45.3)	12 (5.7)	14.61 (7.52-30.40)

Table 3.6 cont.: Modified ISAAC questionnaire administered for one-year-old children

Variable	**Cases (n=214)**	**Controls (n=209)**	**OR (95% CI)**
Has your child been diagnosed with asthma by a doctor in the past year? (n/%)	12 (5.6)	1 (0.5)	12.75 (1.85-547.39)
Has your child received treatment for wheezing or whistling in the chest in the past year? (n/%)	110 (51.4)	12 (5.7)	20.89 (10.69-43.56)
Has your child ever been diagnosed with eczema by a doctor in the past year? (n/%)	41 (19.2)	24 (11.5)	1.88 (1.06-3.4)
Has your child ever had an itchy rash that comes and goes in the past year? (n/%)	85 (39.7)	64 (30.6)	1.49 (0.98-2.28)
Has your child ever had an itchy rash that affects the folds of the elbows, behind the knees, front of the ankles, buttocks, around the eyes during the past year? (n/%)	83 (38.8)	57 (27.3)	1.69 (1.10-2.60)

*ISAAC: International Study of Asthma and Allergies in Childhood; †n: number; ¶ OR: Odds ratio; §CI: Confidence interval; ‡SD: Standard deviation

Univariate regression analyses were performed for clinical pulmonary function outcome variables (ISAAC questions) in one and two-year-old participants. All independent variables with a p-value of <0.2 were included in the multivariate analysis, the aim of which was to establish which factors influence pulmonary health of infants. Outcomes for one-year-old children are reported in tables 3.7-3.9 and supplementary table 3.1, and for two-year-old children in tables 3.10-3.12, and supplementary table 3.2.

On multivariate regression in one and two-year-old children, severe LRTI was independently associated with wheezing or whistling in the chest, wheezing or whistling in the chest during activity, having received treatment for wheezing or whistling in the chest, had any admissions for wheezing or whistling in the chest or any chest infection, or reported the presence of a dry cough at night, apart from when he/she has a cold or an infection during the past year, or after the RSV-LRTI until their first visit for the one-year-old children.

Furthermore one-year-old children were more likely to have experienced wheezing or whistling in the chest if they weighed more at one year, increasing for every 100g increase in weight, and less likely if there was less crowding at home, and more likely to have been hospitalized for a wheezing episode or with a chest infection, if they attended crèche, but less likely to have been admitted for wheezing if they had household pets or had been exclusively breastfed.

No other variables reached significance in the multivariate analysis of two-year-old children.

Table 3.7: Wheezing or whistling in the chest in **one-year-old** children

Independent Variables	Wheeze or whistling in chest		Univariate analysis		Multivariate analysis
	Yes	No	OR* (95% CI†)	p-value	aOR¶ (95% CI)
Birthweight (g) (Median (IQR§), Range)	3240 (2920-3715), 2920-3715	3091 (2860-3370), 2500-4600	1.000 (0.999-1.001)	0.230	
Weight at 1 year (kg) (Median (IQR), Range)	9.9 (9.1-10.9), 7.2-15.0	9.8 (8.8-10.4), 5.4-15.1	**1.167 (1.045-1.304)**	**0.006**	**1.175 (1.008-1.369)**
Height at 1 year (cm) (Median (IQR), Range)	74 (72-77), 66-89	74 (72-77), 54-88	1.027 (0.984-1.070)	0.223	
Gender (Male) (n‡/%)	133 (22.3)	194 (32.5)	0.943 (0.678-1.310)	0.726	
HEU° (n/%)	45 (7.7)	93 (16.0)	**0.677 (0.452-1.013)**	**0.057**	0.743 (0.463-1.263)
Maternal atopy‡ (n/%)	30 (5.1)	43 (7.3)	1.060 (0.644-1.745)	0.819	
Paternal atopy‡ (n/%)	20 (3.5)	28 (4.9)	1.087 (0.597-1.980)	0.786	
Sibling atopy‡ (n/%)	19 (4.9)	20 (5.2)	**1.666 (0.857-3.240)**	**0.132**	1.322 (0.647-2.700)
IETS‡†† exposure (n/%)	11 (1.9)	9 (1.5)	**1.849 (0.754-4.534)**	**0.179**	1.809 (0.989-3.312)
ETS‡¶¶ exposure (n/%)	45 (7.6)	60 (10.1)	1.148 (0.749-1.759)	0.526	
Exclusively breastfed (n/%)	93 (15.6)	215 (36.0)	**0.424 (0.303-0.592)**	**<0.005**	0.647 (0.402-1.039)
Less than 6 people in house (n/%)	129 (21.8)	231 (39.0)	**0.635 (0.454-0.889)**	**0.008**	**0.543 (0.338-0.869)**
Siblings in crèche (n/%)	88 (15.0)	100 (17.0)	**1.366 (1.008-1.852)**	**0.045**	1.167 (0.747-1.822)
Attends crèche (n/%)	59 (10.0)	56 (9.4)	**1.776 (1.178-2.676)**	**0.006**	1.100 (0.598-2.026)
Pets (n/%)	45 (7.6)	56 (9.4)	1.266 (0.822-1.951)	0.284	
Severe RSV-LRTI§§ (n/%)	170 (28.5)	136 (22.8)	**4.022 (2.829-5.718)**	**<0.005**	**2.782 (1.745-4.436)**

*OR: Odds ratio; †CI: Confidence interval; ¶aOR: Adjusted odds ratio; §IQR: Interquartile range; ‡n: number; °HEU: HIV exposed uninfected; ‡Parental reported; ††IETS: Intrauterine environmental tobacco smoke exposure; ¶¶ETS: Environmental tobacco smoke exposure; §§RSV-LRTI: Respiratory syncytial virus lower respiratory tract infection

Table 3.8: Admission for a wheezing episode or whistling in the chest in **one-year-old** children

Independent Variables	**Admission for wheezing**		**Univariate analysis**		**Multivariate analysis**
	Yes	**No**	**OR* (95% CI†)**	**p-value**	**aOR¶ (95% CI)**
Birthweight (g) (Median (IQR§), Range)	3080 (2820-3430), 2510-3900	3120 (2890-3395), 2500-4875	0.999 (0.999-1.000)	**0.136**	0.999 (0.999-1.000)
Weight at 1 year (kg) (Median (IQR), Range)	10.0 (9.3-10.8), 7.4-14.5	9.8 (8.9-10.5), 5.4-15.1	1.156 (1.011-1.321)	**0.034**	1.121 (0.955-1.318)
Height at 1 year (cm) (Median (IQR), Range)	74 (72-77), 66-87	74 (72-77), 54-89)	1.009 (0.957-1.063)	0.743	
Gender (Male) (n‡/%)	66 (11.1)	262 (43.8)	0.794 (0.522-1.207)	0.280	
HEU° (n/%)	22 (3.8)	116 (19.9)	0.852 (0.509-1.427)	0.543	
Maternal atopy‡ (n/%)	13 (2.2)	60 (10.1)	0.957 (0.505-1.813)	0.893	
Paternal atopy‡ (n/%)	6 (1.0)	42 (7.3)	0.611 (0.253-1.478)	0.275	
Sibling atopy‡ (n/%)	6 (1.6)	33 (8.6)	1.133 (0.451-2.847)	0.791	
IETS‡†† exposure (n/%)	5 (0.8)	15 (2.5)	1.471 (0.523-4.138)	0.464	
ETS‡¶¶ exposure (n/%)	20 (3.4)	85 (14.2)	1.037 (0.606-1.775)	0.895	
Exclusively breastfed (n/%)	31 (5.2)	278 (46.5)	0.291 (0.185-0.458)	**<0.005**	**0.430 (0.263-0.705)**
Less than 6 people in house (n/%)	61 (10.3)	300 (50.5)	0.774 (0.490-1.129)	**0.165**	0.752 (0.473-1.194)
Siblings in crèche (n/%)	42 (7.1)	146 (24.8)	1.050 (0.718-1.535)	0.800	
Attends crèche (n/%)	35 (5.9)	81 (13.6)	2.286 (1.434-3.643)	**0.001**	**1.790 (1.068-2.998)**
Pets (n/%)	30 (5.1)	71 (12.0)	2.214 (1.357-3.614)	**0.001**	**2.128 (1.229-3.685)**
Severe RSV-LRTI§§ (n/%)	95 (15.9)	212 (35.5)	7.702 (4.403-13.472)	**<0.005**	**5.656 (3.170-10.092)**

*OR: Odds ratio; †CI: Confidence interval; ¶aOR: Adjusted odds ratio; §IQR: Interquartile range; ‡n: number; °HEU: HIV exposed uninfected; ‡Parental reported; ††IETS: Intrauterine environmental tobacco smoke exposure; ¶¶ETS: Environmental tobacco smoke exposure; §§RSV-LRTI: Respiratory syncytial virus lower respiratory tract infection

Table 3.9: Admission for a chest infection in **one-year-old** children

Independent Variables	**Admission for chest infection**		**Univariate analysis**		**Multivariate analysis**
	Yes	**No**	**OR* (95% CI†)**	**p-value**	**aOR¶ (95% CI)**
Birthweight (g) (Median (IQR§), Range)	3078 (2820-3410), 2510-3900	3125 (2885-3400), 2500-4875	0.999 (0.999-1.000)	**0.156**	0.999 (0.999-1.000)
Weight at 1 year (kg) (Median (IQR), Range)	9.9 (9.2-10.8), 6.7-14.5	9.8 (8.9-10.5), 5.4-15.1	1.070 (0.917-1.249)	0.392	
Height at 1 year (cm) (Median (IQR), Range)	74 (72-77), 65-87	74 (72-77), 54-89	0.995 (0.937-1.057)	0.869	
Gender (Male) (n‡/%)	44 (7.4)	284 (47.5)	0.961 (0.597-1.548)	0.871	
HEU° (n/%)	15 (2.6)	123 (21.1)	0.847 (0.463-1.551)	0.591	
Maternal atopy‡ (n/%)	10 (1.7)	63 (10.6)	1.073 (0.525-2.193)	0.846	
Paternal atopy‡ (n/%)	4 (0.7)	500 (86.8)	0.576 (0.201-1.651)	0.304	
Sibling atopy‡ (n/%)	3 (0.8)	36 (9.3)	0.793 (0.231-2.716)	0.712	
IETS‡†† exposure (n/%)	2 (0.3)	18 (3.0)	0.717 (0.163-3.151)	0.660	
ETS‡¶¶ exposure (n/%)	9 (1.5)	96 (16.1)	0.565 (0.273-1.171)	**0.125**	0.882 (0.392-1.983)
Exclusively breastfed (n/%)	16 (2.7)	293 (49.0)	0.196 (0.110-0.348)	**<0.005**	**0.309 (0.166-0.574)**
Less than 6 people in house (n/%)	47 (7.9)	314 (52.9)	0.940 (0.580-1.524)	0.802	
Siblings in crèche (n/%)	26 (4.4)	162 (27.5)	0.856 (0.541-1.352)	0.505	
Attends crèche (n/%)	27 (4.6)	89 (15.0)	2.485 (1.481-4.172)	**<0.005**	**1.826 (1.036-3.222)**
Pets (n/%)	18 (3.0)	83 (14.0)	1.565 (0.879-2.786)	**0.128**	1.456 (0.768-2.761)
Severe RSV-LRTI§§ (n/%)	73 (12.2)	234 (39.1)	14.818 (6.333-34.675)	**<0.005**	**10.428 (4.390-24.774)**

*OR: Odds ratio; †CI: Confidence interval; ¶aOR: Adjusted odds ratio; §IQR: Interquartile range; ‡n: number; °HEU: HIV exposed uninfected; ‡Parental reported; ††IETS: Intrauterine environmental tobacco smoke exposure; ¶¶ETS: Environmental tobacco smoke exposure; §§RSV-LRTI: Respiratory syncytial virus lower respiratory tract infection

Table 3.10: Wheezing or whistling in the chest in the past year in **two-year-old** children

Independent Variables	Presence of wheeze		Univariate analysis		Multivariate analysis
	Yes	**No**	**OR* (95% CI†)**	**p-value**	**aOR¶ (95% CI)**
Birthweight (g) (Median (IQR§), Range)	3100 (2880-3400), 2515-4600	3175 (2855-3440), 2500-4515	0.999 (0.999-1.000)	0.297	
Weight at 1 year (kg) (Median (IQR), Range)	12.0 (10.7-13.1), 8.7-20.0	11.7 (10.7-12.6), 7.5-20.4	1.038 (0.925-1.165)	0.527	
Height at 1 year (cm) (Median (IQR), Range)	87 (85-90), 72-99	86 (83-89), 70-100	**1.044 (0.999-1.091)**	**0.055**	1.020 (0.957-1.087)
Gender (Male) (n‡/%)	89 (21.2)	120 (28.6)	**0.759 (0.513-1.124)**	**0.169**	0.865 (0.497-1.503)
HEU° (n/%)	42 (10.4)	55 (13.6)	1.223 (0.770-1.943)	0.394	
Maternal atopy‡ (n/%)	15 (3.6)	20 (4.8)	1.168 (0.580-2.352)	0.664	
Paternal atopy‡ (n/%)	10 (2.5)	12 (3.1)	1.290 (0.543-3.063)	0.564	
Sibling atopy‡ (n/%)	20 (7.7)	18 (7.0)	**1.778 (0.890-3.552)**	**0.103**	1.476 (0.680-3.203)
IETS‡†† exposure (n/%)	7 (1.7)	7 (1.7)	1.563 (0.538-4.542)	0.412	
ETS‡¶¶ exposure (/%)	26 (6.2)	32 (7.6)	1.298 (0.742-2.270)	0.361	
Exclusively breastfed (n/%)	98 (23.4)	150 (35.8)	1.014 (0.681-1.511)	0.945	
Less than 6 people in house (n/%)	86 (20.6)	112 (26.8)	**1.370 (0.924-2.031)**	**0.116**	0.899 (0.512-1.579)
Siblings in crèche (n/%)	65 (15.6)	88 (21.1)	1.034 (0.713-1.500)	0.858	
Attends crèche (n/%)	47 (11.2)	92 (22.0)	0.707(0.463-1.081)	**0.110**	0.749 (0.409-1.371)
Pets (n/%)	42 (10.1)	31 (7.5)	**2.495 (1.492-4.174)**	**<0.005**	1.928 (0.960-3.873)
Severe RSV-LRTI§§ (n/%)	131 (31.2)	80 (19.1)	**8.428 (5.317-13.361)**	**<0.005**	**4.649 (2.614-8.269)**

*OR: Odds ratio; †CI: Confidence interval; ¶aOR: Adjusted odds ratio; §IQR: Interquartile range; ‡n: number; °HEU: HIV exposed uninfected; ‡Parental reported; ††IETS: Intrauterine environmental tobacco smoke exposure; ¶¶ETS: Environmental tobacco smoke exposure; §§RSV-LRTI: Respiratory syncytial virus lower respiratory tract infection

Table 3.11: Admission for a wheezing episode or whistling in the chest in **two-year-old** children

Independent Variables	**Admission for wheezing**		**Univariate analysis**		**Multivariate analysis**
	Yes	**No**	**OR* (95% CI†)**	**p-value**	**aOR¶ (95% CI)**
Birthweight (g) (Median (IQR§), Range)	3088 (2870-3395), 2540-4160	3158 (2870-3440), 2500-4600	0.999 (0.999-1.000)	**0.186**	0.999 (0.999-1.000)
Weight at 1 year (kg) (Median (IQR), Range)	12.0 (10.9-13.0), 9.2-20.0	11.8 (10.7-12.8), 7.5-20.4	1.063 (0.935-1.210)	0.350	
Height at 1 year (cm) (Median (IQR), Range)	87 (85-90), 72-99	86 (83-89), 70-100	1.050 (0.998-1.103)	**0.058**	1.042 (0.977-1.111)
Gender (Male) (n‡/%)	55 (13.1)	154 (36.7)	0.802 (0.513-1.255)	0.335	
HEU° (n/%)	25 (6.2)	72 (17.8)	1.133 (0.670-1.918)	0.641	
Maternal atopy‡ (n/%)	11 (2.6)	24 (5.8)	1.482 (0.699-3.143)	0.305	
Paternal atopy‡ (n/%)	5 (1.2)	17 (4.3)	0.858 (0.308-2.388)	0.769	
Sibling atopy‡ (n/%)	12 (4.5)	26 (10.0)	1.538 (0.725-3.264)	0.262	
IETS‡†† exposure (n/%)	4 (1.0)	10 (2.4)	1.253 (0.384-4.085)	0.708	
ETS‡¶¶ exposure (n/%)	16 (3.8)	42 (10.0)	1.218 (0.652-2.275)	0.536	
Exclusively breastfed (n/%)	67 (16.0)	181 (43.2)	1.438 (0.903-2.291)	**0.126**	1.274 (0.729-2.227)
Less than 6 people in house (n/%)	54 (12.9)	144 (34.5)	1.344 (0.859-2.102)	**0.196**	0.865 (0.502-1.488)
Siblings in crèche (n/%)	45 (10.8)	108 (25.9)	1.205 (0.795-1.829)	0.380	
Attends crèche (n/%)	27 (6.5)	112 (26.8)	0.656 (0.399-1.077)	**0.096**	0.849 (0.469-1.536)
Pets (n/%)	22 (5.3)	51 (12.3)	1.430 (0.817-2.504)	0.210	
Severe RSV-LRTI§§ (n/%)	99 (23.6)	112 (26.7)	60.696 (18.811-195.843)	**<0.005**	**59.053 (18.150-192.136)**

*OR: Odds ratio; †CI: Confidence interval; ¶aOR: Adjusted odds ratio; §IQR: Interquartile range; ‡n: number; °HEU: HIV exposed uninfected; ‡Parental reported; ††IETS: Intrauterine environmental tobacco smoke exposure; ¶¶ETS: Environmental tobacco smoke exposure; §§RSV-LRTI: Respiratory syncytial virus lower respiratory tract infection

Table 3.12: Admission for a chest infection in **two-year-old** children

Independent Variables	**Admission for LRTI**		**Univariate analysis**		**Multivariate analysis**
	Yes	**No**	**OR* (95% CI†)**	**p-value**	**aOR¶ (95% CI)**
Birthweight (g) (Median (IQR§), Range)	3098 (2860-3405), 2540-4160	3150 (2870-3435), 2500-4600	0.999 (0.999-1.000)	0.422	
Weight at 1 year (kg) (Median (IQR), Range)	12.2 (10.9-13.4), 9.3-20	11.8 (10.7-12.6), 7.5-20.4	**1.125 (0.977-1.297)**	**0.102**	0.976 (0.771-1.236)
Height at 1 year (cm) (Median (IQR), Range)	88 (85-92), 72-99	86 (83-89), 70-100	**1.096 (1.035-1.164)**	**<0.005**	1.078 (0.970-1.198)
Gender (Male) (n‡/ %)	39 (9.3)	169 (40.3)	0.803 (0.483-1.337)	0.399	
HEU° (n/%)	17 (4.2)	80 (19.9)	0.992 (0.544-1.807)	0.978	
Maternal atopy‡ (n/%)	6 (1.5)	29 (7.0)	1.003 (0.400-2.512)	0.995	
Paternal atopy‡ (n/%)	1 (0.3)	21 (5.3)	**0.201 (0.027-1.521)**	**0.120**	0.154 (0.017-1.418)
Sibling atopy‡ (n/%)	10 (3.9)	28 (10.9)	**2.024 (0.899-4.555)**	**0.089**	1.800 (0.700-4.630)
IETS exposure‡†† (n/%)	3 (0.7)	11 (2.6)	1.324 (0.360-4.871)	0.673	
ETS exposure‡¶¶ (n/%)	10 (2.4)	48 (11.5)	1.001 (0.481-2.087)	0.997	
Exclusively breastfed (n/%)	47 (11.2)	200 (47.9)	1.372 (0.808-2.331)	0.242	
Less than 6 people in house (n/%)	36 (8.6)	162 (38.9)	1.130 (0.680-1.878)	0.638	
Siblings in crèche (n/%)	29 (7.0)	123 (29.6)	0.995 (0.615-1.610)	0.985	
Attends crèche (n/%)	19 (4.6)	120 (28.8)	**0.672 (0.381-1.187)**	**0.171**	0.615 (0.258-1.468)
Pets (n/%)	15 (3.6)	58 (14.0)	1.284 (0.680-2.423)	0.440	
Severe RSV-LRTI§§ (n/%)	69 (16.5)	141 (33.7)	**33.603 (10.371-108.877)**	**<0.005**	**24.912 (5.799-107.017)**

*OR: Odds ratio; †CI: Confidence interval; ¶aOR: Adjusted odds ratio; §IQR: Interquartile range; ‡n: number; °HEU: HIV exposed uninfected; ‡Parental reported; ††IETS: Intrauterine environmental tobacco smoke exposure; ¶¶ETS: Environmental tobacco smoke exposure; §§RSV-LRTI: Respiratory syncytial virus lower respiratory tract infection

3.3 Pulmonary function data following RSV-LRTI during infancy

3.3.1 Pulmonary function success rates in one and two-year-old children

Pulmonary function testing was performed on all 308 and 292 one-year-old cases and controls and all 214 and 209 two-year-old cases and controls respectively, with the successful completion of testing reported in Tables 3.13 and 3.14.

Table 3.13: Pulmonary function success rates for one-year-old children

	Cases (n=308)	**Controls (n=292)**
FOT* completed (n/%)	282 (91.6)	276 (94.5)
TBFVL† completed (n/%)	267 (86.7)	268 (91.8)
MBW¶ completed (n/%)	227 (73.7)	201 (68.8)

*FOT: Forced oscillation technique; †TBFVL: Tidal breath flow-volume loops; ¶MBW: Multiple Breath washout

Table 3.14: Pulmonary function success rates for two-year-old children

	Cases (n=214)	**Controls (n=209)**
FOT* completed (n/%)	194 (90.7)	184 (88.0)
TBFVL† completed (n/%)	190 (88.8)	176 (84.2)
MBW¶ completed (n/%)	171 (79.9)	153 (73.2)

*FOT: Forced oscillation technique; †TBFVL: Tidal breath flow-volume loops; ¶MBW: Multiple Breath washout

3.3.2 Forced oscillation technique data in one and two-year-old children

The FOT indices for cases and controls at one and two-year-old children are presented in Tables 3.15 and 3.16, and Figures 3.1 and 3.2.

The mean resistance was similar in one-year-old cases and controls, but increased in two-year-old cases when compared to controls, however, the intra-breath measurements of resistance (end-expiratory and end-inspiratory) were increased in both one and two-year-old cases, while the difference between end-expiratory and end-inspiratory resistance was increased in one-year-old cases, but similar in two-year-old cases and controls.

The mean reactance was increased in both one and two-year-old cases, as was the intra-breath measurements of reactance (end-inspiratory and end-expiratory reactance), however the difference between the end-expiratory and end-inspiratory reactance was similar in one-year-old cases, but also increased in two-year-old cases.

Linear regression analyses were performed for all relevant FOT outcome variables in one and two-year-old children. All independent variables with a p-value of <0.2 were included in a multivariate linear regression analysis, the aim of which was to establish which factors, including severe RSV-LRTI, influence the pulmonary health of infants, as measured by pulmonary function testing. Mean airway resistance and reactance outcomes are reported in Tables 3.17-3.20. Difference between end-expiratory and end-inspiratory resistance and reactance outcomes are reported in supplementary Tables 3.3-3.6.

In one-year-old children, multivariate linear regression of FOT found no relationship between the mean respiratory system resistance and any of the independent variables included in the model, whereas in two-year-old children there was an association with severe RSV-LRTI and an increase in mean resistance. In both one and two-year-old children severe RSV-LRTI during infancy alone was also associated with a positive increase in the mean respiratory system reactance.

In neither one nor two-year-old children was severe RSV-LRTI associated with the difference between end-expiratory and end-inspiratory resistance, however in two-year-old children it was associated with the difference between end-expiratory and end-inspiratory reactance.

On exploration for correlation between ISAAC questions and the FOT indices using Spearman's rank correlation in one and two-year-old children, either no correlation or at best weak correlations were found.

Table 3.15: Forced oscillation technique data for **one-year-old** children

Variables	**Cases (n*=282)**	**Controls (n=276)**	**p-value**
R_{mean}† (hPa.s.l^{-1}) (Median (IQR¶), Range)	24.70 (20.09;30.33), 12.42-79.60	23.73 (20.09;30.15), 12.00-62.76	0.674
R_{eE}§ (hPa.s.l^{-1}) (Median (IQR), Range)	20.45 (17.23;24.34), 8.76-56.61	18.71 (16.26;21.55), 9.78-56.11	**<0.001**
R_{eI}‡ (hPa.s.l^{-1}) (Median (IQR), Range)	22.77 (19.13;28.45), 11.91-66.07	20.24 (17.11;24.81), 9.39-57.49	**<0.001**
ΔR° (hPa.s.l^{-1}) (Median (IQR), Range)	-2.38 (-5.19;-0.46), -34.93-8.34	-1.98 (-4.29;0.42), -21.49-5.02	**0.029**
$\Delta R/V_T$⁑ (hPa.s.l^{-2}) (Median (IQR), Range)	-26.69 (-60.47;-4.60), -340.35-157.67	-26.08 (-73.78;7.35), -509.21-111.76	0.914
X_{mean}†† (hPa.s.l^{-1}) (Median (IQR), Range)	-3.70 (-6.81;-1.97), -20.11-3.43	-1.81 (-4.70;-0.23), -29.87-3.64	**<0.001**
X_{eE}¶¶ (hPa.s.l^{-1}) (Median (IQR), Range)	-2.40 (-4.32;-0.53), -23.93-6.63	-0.06 (-2.39;1.27), -23.23-5.21	**<0.001**
X_{eI}§§ (hPa.s.l^{-1}) (Median (IQR), Range)	-1.90 (-3.91;-0.53), -18.44-3.72	-0.14 (-1.92;1.17), -34.67-5.41	**<0.001**
ΔX°° (hPa.s.l^{-1}) (Median (IQR), Range)	-0.18 (-1.47;0.84), -20.44-23.45	-0.08 (-1.12;0.94), -21.10-22.50	0.235
$\Delta X/V_T$ (hPa.s.l^{-2}) (Median (IQR), Range)	-2.01 (-15.67;9.79), -248.02-225.84	-1.40 (-16.63;13.33), -182.27-349.04	0.525

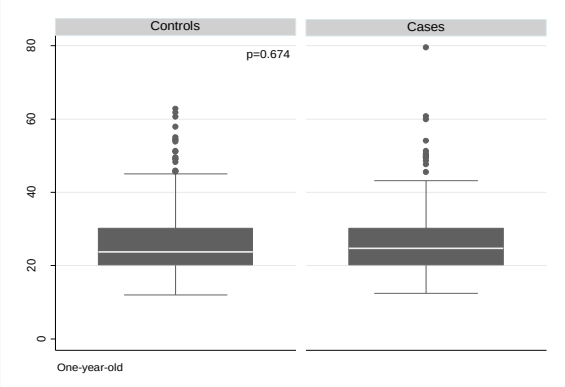
*n: number; †R_{mean}: Mean airway resistance; ¶IQR: Interquartile range; §R_{eE}: End-expiratory airway resistance; ‡R_{eI}: End-inspiratory airway resistance; °ΔR: Difference between end-expiratory and end-inspiratory resistance; ⁑V_T: Tidal volume; ††X_{mean}: Mean airway reactance; ¶¶X_{eE}: End-expiratory airway reactance; §§X_{eI}: End-inspiratory reactance; °°ΔX Difference between end-expiratory and end-inspiratory reactance

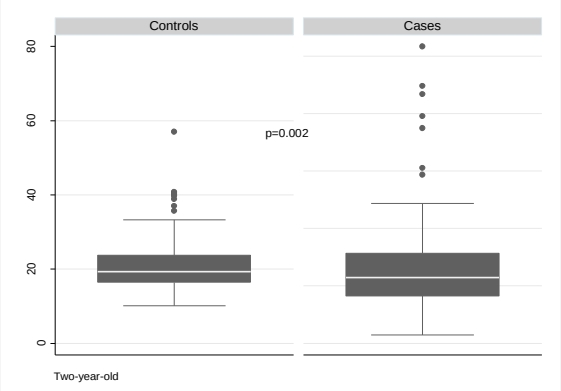
Table 3.16: Forced oscillation technique data for **two-year-old** children

Variables	**Cases (n*=214)**	**Controls (n=209)**	**p-value**
R_{mean}† (hPa.s.l^{-1}) (Median (IQR¶), Range)	21.43 (18.27;25.69), 11.47-61.69	19.29 (16.38;23.77), 10.10-57.09	**0.002**
R_{eE}§ (hPa.s.l^{-1}) (Median (IQR), Range)	16.65 (14.56;19.09), 9.40-42.37	15.10 (13.14;17.37), 8.28-37.19	**<0.001**
R_{eI}‡ (hPa.s.l^{-1}) (Median (IQR), Range)	18.99 (15.79;22.35), 10.17-49.02	17.00 (14.63;20.44), 9.91-40.26	**<0.001**
ΔR° (hPa.s.l^{-1}) (Median (IQR), Range)	-2.13 (-1.11;-0.50), -12.44-3.53	-1.69 (-3.58;-0.41), -15.93-3.48	0.382
$\Delta R/V_T$⁑ (hPa.s.l^{-2}) (Median (IQR), Range)	-17.07 (-30.70;-4.36), -157.47-39.34	-13.98 (-29.17;-4.30), -158.51-32.13	0.388
X_{mean}†† (hPa.s.l^{-1}) (Median (IQR), Range)	-0.49 (-2.25;0.75), -10.67-2.76	0.74 (-0.63;1.59), -9.18-4.07	**<0.001**
X_{eE}¶¶ (hPa.s.l^{-1}) (Median (IQR), Range)	0.57 (-1.01;1.57), -12.81-3.90	1.69 (0.79;2.35), -7.40-4.41	**<0.001**
X_{eI}§§ (hPa.s.l^{-1}) (Median (IQR), Range)	0.72 (-0.45;1.51), -5.52-5.47	1.38 (0.53;2.17), -6.64-4.44	**<0.001**
ΔX°° (hPa.s.l^{-1}) (Median (IQR), Range)	-0.19 (-0.79;0.51), -10.09-4.25)	0.12 (-0.45;0.69), -6.31-5.67	**0.001**
$\Delta X/V_T$ (hPa.s.l^{-2}) (Median (IQR), Range)	-1.51 (-6.71-3.90), -78.13-42.23	1.49 (-3.57-6.54), -64.02-56.47	**<0.001**

*n: number; †R_{mean}: Mean airway resistance; ¶IQR: Interquartile range; §R_{eE}: End-expiratory airway resistance; ‡R_{eI}: End-inspiratory airway resistance; °ΔR: Difference between end-expiratory and end-inspiratory resistance; ⁑V_T: Tidal volume; ††X_{mean}: Mean airway reactance; ¶¶X_{eE}: End-expiratory airway reactance; §§X_{eI}: End-inspiratory reactance; °°ΔX Difference between end-expiratory and end-inspiratory reactance

Figure 3.1: Mean airway resistance in **one and two-year-old** children

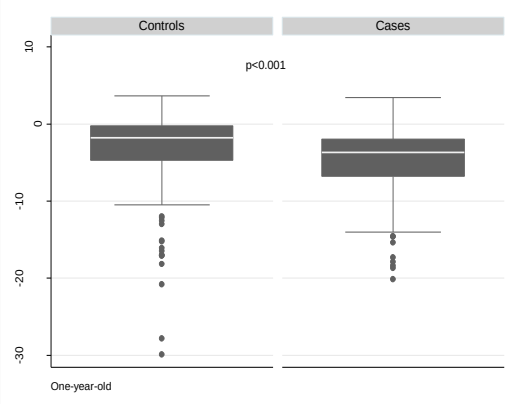


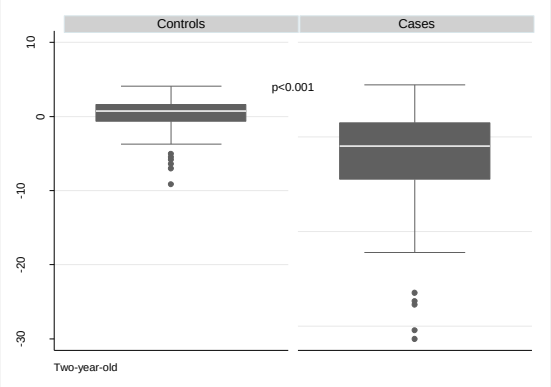


R_{Mean}: Mean airway resistance ($hPa.s.l^{-1}$)

R_{Mean}: Mean airway resistance ($hPa.s.l^{-1}$)

Figure 3.2: Mean airway reactance in **one and two-year-old** children





X_{Mean}: Mean airway reactance ($hPa.s.l^{-1}$)

X_{Mean}: Mean airway reactance ($hPa.s.l^{-1}$)

Table 3.17: Mean airway resistance in **one-year-old** children

R_{mean}*	**Univariate analysis**		**Multivariate analysis**	
Independent Variables	**Coefficient (95% CI†)**	**p-value**	**Coefficient (95% CI)**	**p-value**
Birthweight (g)	0.000 (-0.002;0.002)	0.740		
Weight at 1 year (kg)	0.142 (-0.466;0.550)	0.871		
Height at 1 year (cm)	-0.044 (-0.239;0.152)	0.661		
Gender (Male)	0.173 (-1.363;1.710)	0.825		
HEU¶	-1.065 (-2.877;0.747)	0.249		
Maternal atopy§	0.067 (-2.222;2.356)	0.954		
Paternal atopy§	0.795 (-1.897;3.486)	0.562		
Sibling atopy§	-0.943 (-3.965;2.079)	0.540		
IETS‡ exposure§	-0.299 (-4.507;3.908)	0.889		
ETS° exposure§	-0.654 (-2.643;1.334)	0.518		
Exclusively breastfed	-1.256 (-2.778;0.267)	**0.106**	-1.347 (-2.833;0.140)	0.076
Less than 6 people in house	0.420 (-1.149;1.988)	0.599		
Siblings in crèche	-0.517 (-1.940;0.905)	0.475		
Attends crèche	1.473 (-0.393;3.339)	**0.122**	1.337 (-0.532;3.205)	0.160
Pets	-0.293 (-2.318;1.732)	0.776		
Severe RSV-LRTI‡‡	-0.009 (-1.534;1.515)	0.990		

*R_{Mean}: Mean airway resistance; †CI: Confidence interval; ¶HEU: HIV exposed uninfected; §Parental reported; ‡IETS: Intrauterine environmental tobacco smoke exposure; °ETS: Environmental tobacco exposure; ‡‡RSV-LRTI: Respiratory syncytial virus lower respiratory tract infection

Table 3.18: Mean airway reactance in **one-year-old** children

X_{mean}*	Univariate analysis		Multivariate analysis	
Independent Variables	**Coefficient (95% CI†)**	**p-value**	**Coefficient (95% CI)**	**p-value**
Birthweight (g)	0.001 (-0.000;0.002)	**0.178**	0.000 (-0.001; 0.001)	0.659
Weight at 1 year (kg)	0.071 (-0.168;0.310)	0.561		
Height at 1 year (cm)	0.076 (-0.016;0.167)	**0.104**	0.086 (-0.010;0.183)	0.078
Gender (Male)	-0.054 (-0.777;0.669)	0.884		
HEU¶	0.074 (-0.794;0.942)	0.868		
Maternal atopy§	0.953 (-0.129;2.036)	**0.084**	0.731 (-0.356;1.818)	0.187
Paternal atopy§	0.631 (-0.636;1.900)	0.328		
Sibling atopy§	0.131 (-1.342;1.604)	0.861		
IETS‡ exposure§	0.122 (-1.861;2.104)	0.904		
ETS° exposure§	0.493 (-0.442;1.429)	0.301		
Exclusively breastfed	0.649 (-0.068; 1.365)	**0.076**	0.327 (-0.425;1.079)	0.393
Less than 6 people in house	0.620 (-0.116;1.357)	**0.099**	0.560 (-0.190; 1.309)	0.143
Siblings in crèche	-0.480 (-1.149;0.188)	**0.159**	-0.024 (-0.758;0.710)	0.948
Attends crèche	-1.149 (-2.052;-0.247)	**0.013**	-0.885 (-1.882;0.113)	0.082
Pets	0.752 (-0.200;1.705)	**0.121**	0.824 (-0.127;1.775)	0.089
Severe RSV-LRTI‡‡	-1.499 (-2.206;-0.793)	**<0.005**	**-1.264 (-2.028; -0.501)**	**<0.001**

*X_{Mean}: Mean airway reactance; †CI: Confidence interval; ¶HEU: HIV exposed uninfected; §Parental reported; ‡IETS: Intrauterine environmental tobacco smoke exposure; °ETS: Environmental tobacco exposure; ‡‡RSV-LRTI: Respiratory syncytial virus lower respiratory tract infection

Table 3.19: Mean airway resistance in **two-year-old** children

R_{mean}*	**Univariate analysis**		**Multivariate analysis**	
Independent Variables	**Coefficient (95% CI†)**	**p-value**	**Coefficient (95% CI)**	**p-value**
Birthweight (g)	-0.002 (-0.004;0.000)	**0.072**	-0.002 (-0.004;0.000)	0.058
Weight at 1 year (kg)	0.112 (-0.310;0.534)	0.603		
Height at 1 year (cm)	-0.043 (-0.200;0.115)	0.595		
Gender (Male)	-0.207 (-1.609;1.195)	0.771		
HEU¶	-0.457 (-2.076;1.162)	0.579		
Maternal atopy§	0.230 (-2.371;2.830)	0.862		
Paternal atopy§	2.073 (-1.208;5.353)	0.215		
Sibling atopy§	-0.972 (-3.368;1.424)	0.425		
IETS‡ exposure§	-0.747 (-4.597;3.103)	0.703		
ETS° exposure§	-1.869 (-3.915;0.176)	**0.073**	-1.797 (-3.811;0.217)	0.080
Exclusively breastfed	0.090 (-1.348;1.529)	0.902		
Less than 6 people in house	-0.479 (-1.890;0.932)	0.505		
Siblings in crèche	0.418 (-0.908;1.743)	0.536		
Attends crèche	1.773 (0.292;3.254)	**0.019**	**2.006 (0.535;3.476)**	**0.008**
Pets	0.731 (-1.080; 2.542)	0.428		
Severe RSV LRTI‡‡	1.834 (0.444;3.224)	**0.010**	**1.898 (0.510;3.286)**	**0.007**

*R_{Mean}: Mean airway resistance; †CI: Confidence interval; ¶HEU: HIV exposed uninfected; §Parental reported; ‡IETS: Intrauterine environmental tobacco smoke exposure; °ETS: Environmental tobacco exposure; ‡‡RSV-LRTI: Respiratory syncytial virus lower respiratory tract infection

Table 3.20: Mean airway reactance in **two-year-old** children

X_{mean}*	Univariate analysis		Multivariate analysis	
Independent Variables	**Coefficient (95% CI†)**	**p-value**	**Coefficient (95% CI)**	**p-value**
Birthweight (g)	0.000 (-0.000; 0.001)	0.211		
Weight at 1 year (kg)	0.061 (-0.080;0.202)	0.393		
Height at 1 year (cm)	0.036 (-0.016;0.087)	**0.174**	0.047 (-0.004;0.099)	0.071
Gender (Male)	-0.038 (-0.506;0.431)	0.874		
HEU¶	-0.062 (-0624;0,500)	0.828		
Maternal atopy§	-0.679 (-1.545;0.186)	**0.124**	-0.538 (-1.369;0.294)	0.205
Paternal atopy§	-0.431 (-1.497;0.635)	0.427		
Sibling atopy§	-0.400 (-1.301;0.501)	0.383		
IETS‡ exposure§	0.195 (-1.091;1.482)	0.765		
ETS° exposure§	-0.306 (-0.991;0.380)	0.381		
Exclusively breastfed	-0.128 (-0.608;0.353)	0.601		
Less than 6 people in house	-0.249 (-0.719;0.222)	0.300		
Siblings in crèche	0.106 (-0.336;0.549)	0.638		
Attends crèche	0.068 (-0.430;0.567)	0.788		
Pets	-0.277 (-0.881;0.327)	0.369		
Severe RSV LRTI‡‡	-1.310 (-1.759;-0.860)	**<0.005**	**-1.326 (-1.779;-0.873)**	**<0.005**

*X_{Mean}: Mean airway reactance; †CI: Confidence interval; ¶HEU: HIV exposed uninfected; §Parental reported; ‡IETS: Intrauterine environmental tobacco smoke exposure; °ETS: Environmental tobacco exposure; ‡‡RSV-LRTI: Respiratory syncytial virus lower respiratory tract infection

3.3.3 Tidal Breath Flow-Volume Loop data in one and two-year-old children

The TBFVL indices for cases and controls in one and two-year-old children are presented in Tables 3.21 and 3.22 and Figures 3.3-3.8, as well as in Supplementary tables 3.7 and 3.8.

One-year-old cases had an increased respiratory rate and a decreased V_T/kg (although the total V_T was similar), with a subsequent increased minute ventilation, and although the respiratory rate and the V_T/kg were similar between two-year-old cases and controls, the minute ventilation remained increased.

The inspiratory flow rates and times were similar between cases and controls in one-year-old children, however there were multiple differences between in expiratory flow rates. In two-year-old children there were multiple differences in both inspiratory and expiratory flow rates and times.

Linear regression analyses were performed for all relevant TBFVL outcome variables in one and two-year-old children. All independent variables with a p-value of <0.2 were included in the multivariate linear regression analysis, the aim of which was to establish which factors, including severe RSV-LRTI, influence the pulmonary health of the infants, as measured by pulmonary function testing. Outcomes are reported in Tables 3.23-3.30 and Supplementary tables 3.9-3.17.

In one-year-old children, severe RSV-LRTI was independently associated with an increased respiratory rate and a decrease in tidal volume (ml/kg), and a decrease in inspiratory time, whereas in both one and two-year-old children severe RSV-LRTI was associated with a decreased expiratory time, an increased mean and peak tidal expiratory flow, as well as a lower $TPEF/T_E$.

On exploration of correlation using Spearman's rank correlation either no or very weak correlation was found between the ISAAC questions and the TBFVL indices, in both one and two-year-old children.

Table 3.21: Selected Tidal Breath Flow-Volume Loop data for **one-year-old** children

Variables	**Cases (n*=267)**	**Controls (n=268)**	**p-value**
Volumes and Times			
Respiratory rate (b/min) (Median (IQR†), Range)	28.65 (25.42;32.09), 18.84-46.84	27.16 (24.40;30.62), 17.33-51.23	<**0.001**
Tidal volume (ml) (Median (IQR), Range)	95.76 (86.49;106.68), 42.87-141.08	97.98 (89.90;107.80), 51.86-154.56	0.124
Tidal volume (ml/kg) (Mean (SD¶), Range)	9.88 (1.77), 5.26-15.71	10.22 (1.8), 5.34-14.13	**0.016**
Minute volume (ml/min) (Median (IQR), Range)	2790 (2550;3014), 1276-4170	2693 (2465;2918), 1528-4005	**0.002**
Total breath time (T_{Tot})(sec) (Median (IQR), Range)	2.11 (1.90;2.39), 1.29-3.32	2.22 (1.97;2.46), 1.18-3.47	**0.001**
Inspiratory time (T_I)(sec) (Median (IQR), Range)	0.96 (0.86;1.07), 0.66-1.72	1.00 (0.90;1.10), 0.59-1.56	**0.012**
Expiratory time (T_E)(sec) (Mean (SD), Range)	1.15 (0.24), 0.63-1.88	1.22 (0.23), 0.59-2.01	**0.001**
T_I/T_{Tot} (%) (Mean (SD), Range)	46.17 (4.34), 34.87-59.04	45.40 (3.64), 35.52-57.38	**0.025**
T_E/T_{Tot} (%) (Mean (SD), Range)	53.83 (4.34), 40.96-65.13	54.60 (3.64), 42.62-64.48	**0.025**
T_I/T_E (%) (Median (IQR), Range)	86.33 (76.40;97.78), 53.69-147.49	83.23 (75.57;89.71), 55.24-135.97	**0.008**
Flows			
MIF§ (ml/s) (Median (IQR), Range)	100.19 (89.20;109.30), 46.68-139.57	96.83 (89.21;105.05), 54.65-156.62	0.167
PIF§ (ml/s) (Median (IQR), Range)	130.63 (114.82;143.37), 57.91-191.81	126.88 (115.79;138.84), 70.06-206.69	0.259
MEF‡ (ml/s) (Median (IQR), Range)	88.50 (78.47;98.28), 38.28-144.38	83.27 (74.52;93.05), 47.55-130.56	<**0.001**
PEF‡ (ml/s) (Median (IQR), Range)	130.42 (114.49;144.17), 50.80-252.05	119.07 (105.04;133.93), 70.06-196.31	<**0.001**
TPIF° (sec) (Median (IQR), Range)	0.48 (0.41;0.56), 0.25-0.89	0.50 (0.42;0.57), 0.25-0.96	0.128
TPEF° (sec) (Median (IQR), Range)	0.38 (0.32;0.48), 0.17-1.65	0.44 (0.35;0.57), 0.16-1.19	<**0.001**
$TPEF/T_E$ (%) (Median (IQR), Range)	35.09 (28.00;41.52), 15.36-97.05	36.65 (30.26;46.48), 14.99-80.68	**0.005**
TEF_{75}‡ (ml/sec) (Median (IQR), Range)	121.70 (106.57;137.83), 46.36-251.83	111.98 (97.40;127.37), 63.69-192.32	<**0.001**

*n: number; †CI: Confidence interval; ¶SD: Standard deviation; §M + PIF Mean and peak tidal inspiratory flow; ‡M + PEF: Mean and peak tidal expiratory flow; °TPIF + PEF: Time to peak tidal inspiratory and expiratory flow; ‡TEF_x Flow at X% of tidal volume remaining;

Table 3.22: Selected Tidal Breath Flow-Volume Loop data for **two-year-old** children

Variables	**Cases (n*=214)**	**Controls (=209)**	**p-value**
Volumes and Times			
Respiratory rate (b/min) (Median (IQR†), Range)	24.67 (22.43-27.52), 15.77-42.39	24.39 (22.34-26.92), 16.75-40.63	0.388
Tidal volume (ml) (Median (IQR), Range)	126.61 (115.76-138.49), 71.74-188.79	122.39 (113.66-132.97), 83.23-189.03	**0.031**
Tidal volume (ml/kg) (Mean (SD¶), Range)	10.55 (1.57), 6.45-14.64	10.74 (1.60), 6.81-16.85	0.357
Minute volume (ml/min) (Median (IQR), Range)	3130 (2918-3397), 2187-4958	3012 (2767-3308), 2043-3899	**0.001**
Total breath time (T_{Tot})(sec) (Median (IQR), Range)	2.44 (0.39), 1.42-3.81	2.48 (0.35), 1.49-3.73	0.344
Inspiratory time (T_I)(sec) (Median (IQR), Range)	1.10 (0.99-1.20), 0.69-1.80	1.10 (1.00-1.19), 0.79-1.77	0.874
Expiratory time (T_E)(sec) (Mean (SD), Range)	1.33 (0.25), 0.70-2.02	1.37 (0.23), 0.70-1.96	0.108
T_I/T_{Tot} (%) (Mean (SD), Range)	45.65 (42.89-48.49), 37.81-58.60	44.55 (42.77-46.88), 38.50-59.37	**0.041**
T_E/T_{Tot} (%) (Mean (SD), Range)	54.35 (51.51-57.11), 41.40-62.19	55.45 (53.12-57.23), 40.63-61.50	**0.041**
T_I/T_E (%) (Median (IQR), Range)	84.34 (75.53-94.53), 61.09-142.96	80.91 (74.86-88.69), 62.95-147.31	**0.038**
Flows			
MIF§ (ml/s) (Median (IQR), Range)	115.01 (16.51), 67.78-176.85	110.71 (13.77), 71.96-143.45	**0.007**
PIF§ (ml/s) (Median (IQR), Range)	148.56 (22.92), 102.47-220.82	142.64 (19.24), 85.35-199.49	**0.008**
MEF‡ (ml/s) (Median (IQR), Range)	98.02 (87.82-108.58), 65.37-173.77	93.20 (85.37-102.68), 63.42-133.86	**0.002**
PEF‡ (ml/s) (Median (IQR), Range)	138.59 (125.67-154.55), 85.22-235.88	128.59 (116.11-142.72), 87.96-179.15	**<0.001**
TPIF° (sec) (Median (IQR), Range)	0.53 (0.43-0.63), 0.24-0.94	0.53 (0.45-0.63),0.29-0.86	0.552
TPEF° (sec) (Median (IQR), Range)	0.45 (0.39-0.56), 0.20-0.98	0.52 (0.41-0.63), 0.27-1.35	**<0.001**
$TPEF/T_E$ (%) (Median (IQR), Range)	35.33 (30.03-42.20), 17.58-62.47	38.87 (32.73-45.32), 19.20-78.20	**0.004**
TEF_{75}⁑ (ml/sec) (Median (IQR), Range)	131.94 (119.03-148.75), 79.08-223.81	119.37 (106.24-134.75), 64.51-175.12	**<0.001**

*n: number; †CI: Confidence interval; ¶SD: Standard deviation; §M + PIF Mean and peak tidal inspiratory flow; ‡M + PEF: Mean and peak tidal expiratory flow; °TPIF + PEF: Time to peak tidal inspiratory and expiratory flow; ⁑TEF_x Flow at X% of tidal volume remaining;

Figure 3.3: Respiratory rate in **one and two-year-old** children

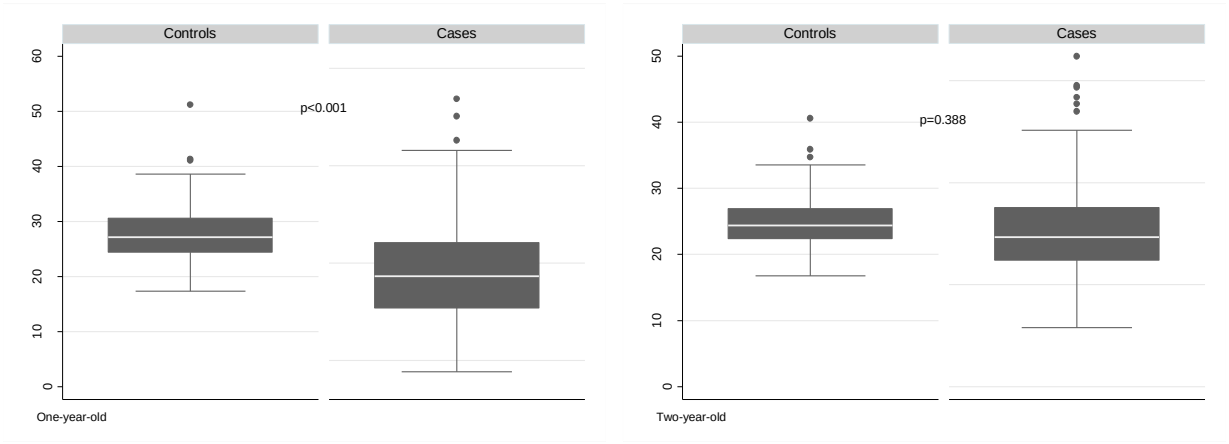


Figure 3.4: Tidal volume in **one and two-year-old** children

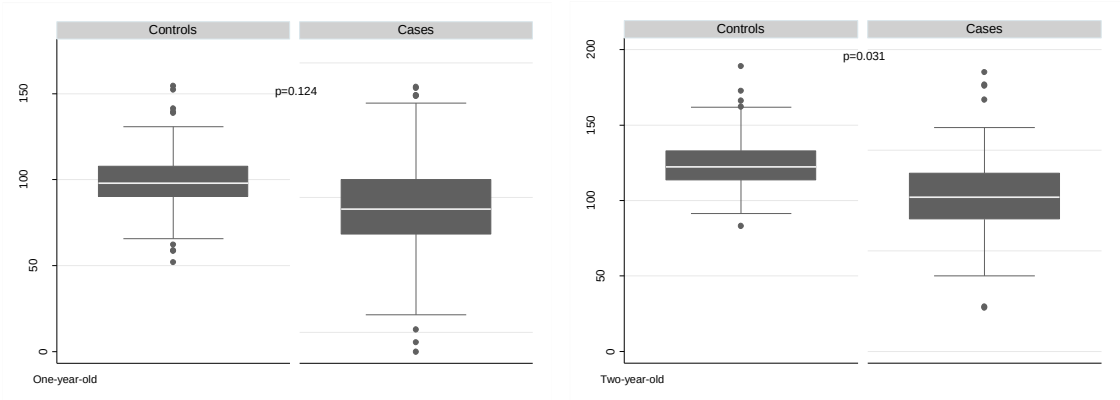


Figure 3.5: Inspiratory and expiratory times in **one and two-year-old** children

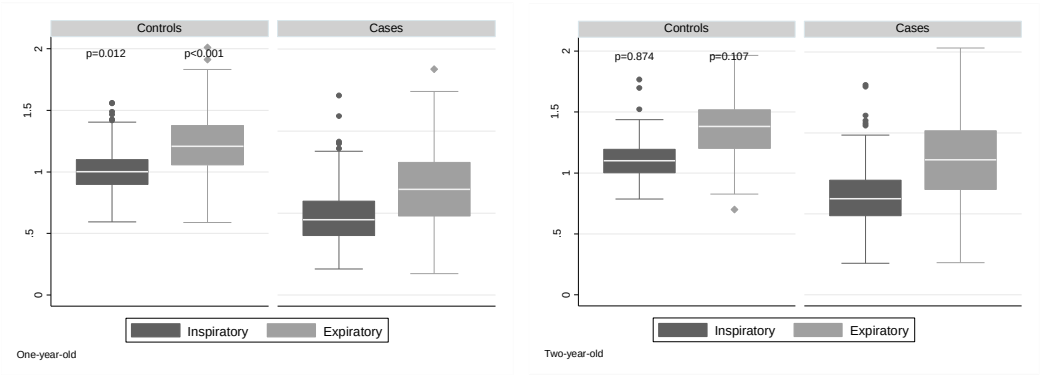


Figure 3.6: Mean tidal inspiratory and expiratory flows in **one and two-year old** children

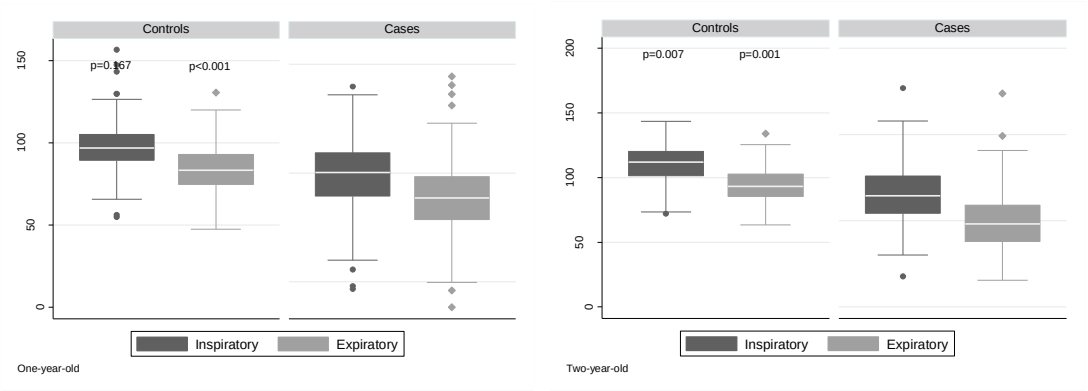


Figure 3.7: Peak tidal inspiratory and expiratory flows in **one and two-year-old** children

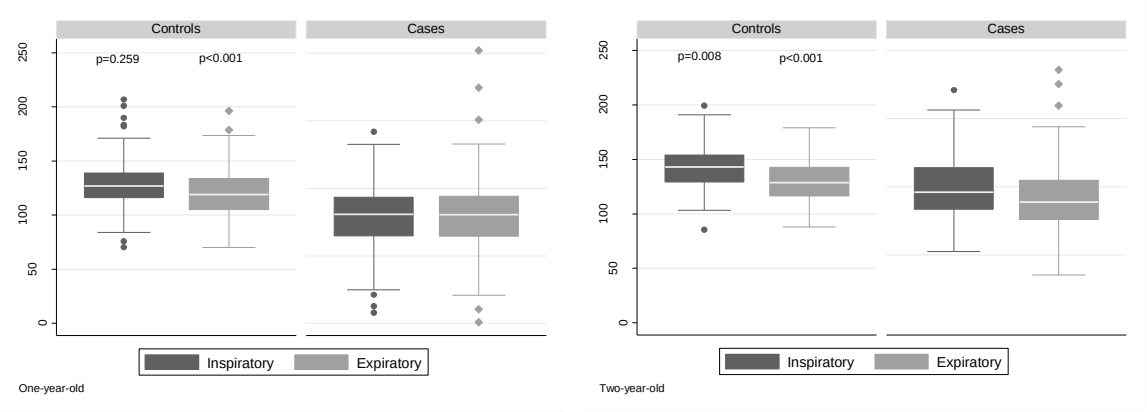


Figure 3.8: Time to peak tidal expiratory flow/Expiratory time in **one and two-year-old** children

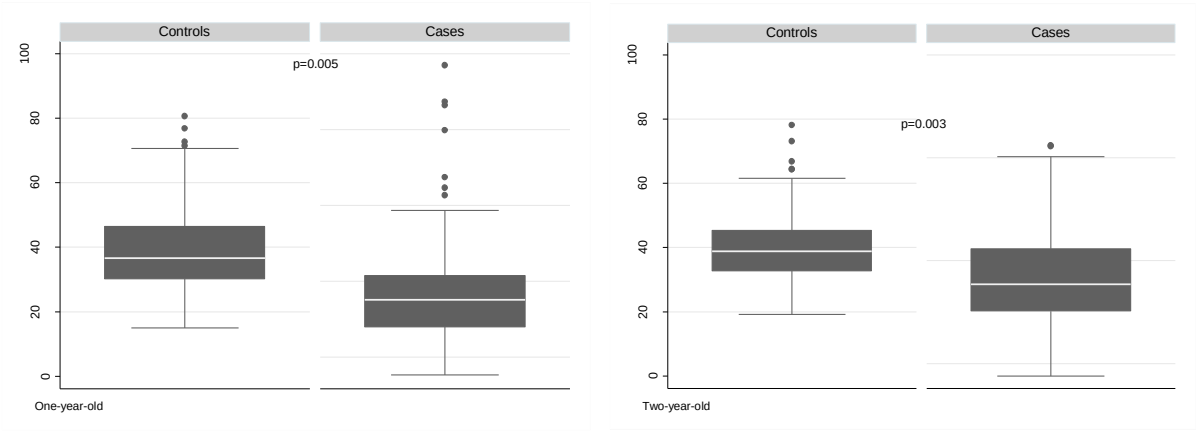


Table 3.23: Respiratory rate in **one-year-old** children

Respiratory rate	**Univariate analysis**		**Multivariate analysis**	
Independent Variables	**Coefficient (95% CI*)**	**p-value**	**Coefficient (95% CI)**	**p-value**
Birthweight (g)	-0.001 (-0.002;-0.000)	**0.018**	-0.001 (-0.002;0.001)	0.274
Weight at 1 year (kg)	-0.158 (-0.432;0.117)	0.260		
Height at 1 year (cm)	-0.202 (-0.303;-0.010)	**<0.005**	**-0.200 (-0.304;-0.096)**	**<0.005**
Gender (Male)	0.072 (-0.754;0.899)	0.864		
HEU†	0.470 (-0.502;1.442)	0.343		
Maternal atopy¶	0.565 (-0.667;1.797)	0.368		
Paternal atopy¶	-0.488 (-1.910;0.934)	0.500		
Sibling atopy¶	0.731 (-0.870;2.331)	0.370		
IETS§ exposure¶	0.654 (-1.617;2.926)	0.572		
ETS‡ exposure¶	0.076 (-0.988;1.140)	0.888		
Exclusively breastfed	-0.605 (-1.424;0.213)	**0.147**	-0.309 (-1.139;0.521)	0.465
Less than 6 people in house	-0.226 (-1.066;0.615)	0.598		
Siblings in crèche	0.236 (-0.529;1.001)	0.545		
Attends crèche	0.667 (-0.374;1.709)	0.209		
Pets	-0.697 (-1.774;0.381)	0.205		
Severe RSV-LRTI°	1.489 (0.679;2.299)	**<0.005**	**1.463 (0.631;2.295)**	**<0.005**

*CI: Confidence interval; †HEU: HIV exposed uninfected; ¶Parental reported; §IETS: Intrauterine environmental tobacco smoke exposure; ‡ETS: Environmental tobacco exposure; °RSV-LRTI: Respiratory syncytial virus lower respiratory tract infection

Table 3.24: Expiratory time in **one-year-old** children

Expiratory time (s)	**Univariate analysis**		**Multivariate analysis**	
Independent Variables	**Coefficient (95% CI*)**	**p-value**	**Coefficient (95% CI)**	**p-value**
Birthweight (g)	0.000 (-0.000;0.000)	**0.071**	0.000 (-0.000;0.000)	0.658
Weight at 1 year (kg)	0.003 (-0.010;0.016)	0.649		
Height at 1 year (cm)	0.010 (0.005;0.145)	**<0.005**	**0.010 (0.005;0.015)**	**<0.005**
Gender (Male)	0.008 (-0.032;0.048)	0.696		
HEU†	-0.018 (-0.065;0.029)	0.449		
Maternal atopy¶	-0.036 (-0.096;0.023)	0.232		
Paternal atopy¶	0.042 (-0.028;0.112)	0.236		
Sibling atopy¶	-0.045 (-0123;0.034)	0.262		
IETS§ exposure¶	-0.011 (-0.121;0.099)	0.841		
ETS‡ exposure¶	0.008 (-0.043;0.060)	0.757		
Exclusively breastfed	0.033 (-0.007;0.072)	**0.107**	0.181 (-0.022;0.058)	0.378
Less than 6 people in house	0.001 (-0.033;0.049)	0.706		
Siblings in crèche	-0.012 (-0.049;0.025)	0.536		
Attends crèche	-0.051 (-0.102;-0.001)	**0.045**	-0.042 (-0.092;0.008)	0.097
Pets	0.019 (-0.033;0.071)	0.479		
Severe RSV-LRTI°	-0.073 (-0.113;-0.034)	**<0.005**	**0.429 (0.058;0.800)**	**0.024**

*CI: Confidence interval; †HEU: HIV exposed uninfected; ¶Parental reported; §IETS: Intrauterine environmental tobacco smoke exposure; ‡ETS: Environmental tobacco exposure; °RSV-LRTI: Respiratory syncytial virus lower respiratory tract infection

Table 3.25: Peak tidal expiratory flow in **one-year-old** children

Peak tidal expiratory flow (ml/s)	**Univariate analysis**		**Multivariate analysis**	
Independent Variables	**Coefficient (95% CI*)**	**p-value**	**Coefficient (95% CI)**	**p-value**
Birthweight (g)	0.005 (0.000;0.011)	**0.038**	0.001 (-0.005;0.006)	0.803
Weight at 1 year (kg)	4.499 (3.231;5.766)	**<0.005**	4.006 (2.448;5.564)	**<0.005**
Height at 1 year (cm)	0.943 (0.454;1.431)	**<0.005**	-0.123 (-0.689;0.442)	0.669
Gender (Male)	-9.037 (-12.947;-5.128)	**<0.005**	-6.513 (-10.376;-2.649)	**<0.005**
HEU†	-2.329 (-7.027;2.369)	0.331		
Maternal atopy¶	0.058 (-5.889;6.005)	0.985		
Paternal atopy¶	2.203 (-4.906;9.313)	0.543		
Sibling atopy¶	4.478 (-3.462;12.418)	0.268		
IETS§ exposure¶	-4.045 (-14.974;6.884)	0.467		
ETS‡ exposure¶	-1.670 (-6.796;3.457)	0.523		
Exclusively breastfed	-5.705 (-9.625;-1.785)	**0.004**	-2.114 (-6.001;1.772)	0.286
Less than 6 people in house	-2.268 (-6.322;1.785)	0.272		
Siblings in crèche	1.514 (-2.179;5.207)	0.421		
Attends crèche	3.750 (-1.279;8.778)	**0.144**	0.956 (-3.861;5.773)	0.697
Pets	-1.433 (-6.604;3.739)	0.587		
Severe RSV-LRTI°	8.852 (4.977;12.727)	**<0.005**	**7.336 (3.418;11.253)**	**<0.005**

*CI: Confidence interval; †HEU: HIV exposed uninfected; ¶Parental reported; §IETS: Intrauterine environmental tobacco smoke exposure; ‡ETS: Environmental tobacco exposure; °RSV-LRTI: Respiratory syncytial virus lower respiratory tract infection

Table 3.26: Time to peak tidal expiratory flow / expiratory time in **one-year-old** children

Time to peak tidal expiratory flow / expiratory time	**Univariate analysis**		**Multivariate analysis**	
Independent Variables	**Coefficient (95% CI*)**	**p-value**	**Coefficient (95% CI)**	**p-value**
Birthweight (g)	-0.001 (-0.003;0.002)	0.700		
Weight at 1 year (kg)	-0.666 (-1.343;0.011)	**0.054**	-0.485 (-1.162;0.192)	0.160
Height at 1 year (cm)	-0.076 (-0.330;0.178)	0.557		
Gender (Male)	1.421 (-0.620;3.461)	**0.172**	0.839 (-1.210;2.888)	0.422
HEU†	1.961 (-0.393;4.325)	**0.102**	1.698 (-0.642;4.038)	0.155
Maternal atopy¶	1.562 (-1.480;4.605)	0.314		
Paternal atopy¶	-0.027 (-3.654;3.600)	0.989		
Sibling atopy¶	-2.392 (-6.630;1.846)	0.268		
IETS§ exposure¶	2.853 (-2.769;8.475)	0.319		
ETS‡ exposure¶	-0.380 (-3.011;2.250)	0.776		
Exclusively breastfed	1.050 (-0.975;3.075)	0.309		
Less than 6 people in house	0.622 (-1.458;2.701)	0.557		
Siblings in crèche	-0.554 (-2.443;1.335)	0.565		
Attends crèche	-0.383 (-2.970;2.204)	0.771		
Pets	0.680 (-1.987;3.347)	0.617		
Severe RSV-LRTI°	-2.993 (-5.002;-0.984)	**<0.005**	**-3.102 (-5.097;-1.107)**	**<0.005**

*CI: Confidence interval; †HEU: HIV exposed uninfected; ¶Parental reported; §IETS: Intrauterine environmental tobacco smoke exposure; ‡ETS: Environmental tobacco exposure; °RSV-LRTI: Respiratory syncytial virus lower respiratory tract infection

Table 3.27: Respiratory rate in **two-year-old** children

Respiratory rate	**Univariate analysis**		**Multivariate analysis**	
Independent Variables	**Coefficient (95% CI*)**	**p-value**	**Coefficient (95% CI)**	**p-value**
Birthweight (g)	-0.000 (-0.002;0.001)	0.382		
Weight at 1 year (kg)	-0.182 (-0.431;0.067)	**0.151**	-0.037 (-0.326;0.252)	0.802
Height at 1 year (cm)	-0.138 (-0.226;-0.051)	**<0.005**	**-0.128 (-0.228;-0.028)**	**0.012**
Gender (Male)	0.826 (-0.007;1.659)	**0.052**	0.688 (-0.131;1.506)	0.099
HEU†	0.170 (-0.832;1.173)	0.739		
Maternal atopy¶	0.025 (-1.439;1.488)	0.974		
Paternal atopy¶	0.395 (-1.552;2.342)	0.690		
Sibling atopy¶	0.378 (-1.148;1.905)	0.626		
IETS§ exposure¶	4.290 (1.762;6.817)	**<0.005**	**3.995 (1.498;6.493)**	**<0.005**
ETS‡ exposure¶	0.532 (-0.707;1.770)	0.399		
Exclusively breastfed	-0.298 (-1.158;0.561)	0.495		
Less than 6 people in house	-0.310 (-1.151;0.530)	0.468		
Siblings in crèche	-0.046 (-0.828;0.739)	0.909		
Attends crèche	0.811 (-0.087;1.708)	**0.076**	0.756 (-1.119;1.631)	0.090
Pets	-0.276 (-1.393;0.842)	0.628		
Severe RSV-LRTI°	0.520 (-0.316;1.356)	0.222		

*CI: Confidence interval; †HEU: HIV exposed uninfected; ¶Parental reported; §IETS: Intrauterine environmental tobacco smoke exposure; ‡ETS: Environmental tobacco exposure; °RSV-LRTI: Respiratory syncytial virus lower respiratory tract infection

Table 3.28: Expiratory time in **two-year-old** children

Expiratory time (s)	**Univariate analysis**		**Multivariate analysis**	
Independent Variables	**Coefficient (95% CI*)**	**p-value**	**Coefficient (95% CI)**	**p-value**
Birthweight (g)	0.000 (-0.000;0.000)	0.512		
Weight at 1 year (kg)	0.007 (-0.008;0.022)	0.353		
Height at 1 year (cm)	0.007 (0.001;0.012)	**0.014**	**0.008 (0.002;0.013)**	**0.005**
Gender (Male)	-0.028 (-0.078;0.022)	0.271		
HEU†	0.002 (-0.058;0.061)	0.957		
Maternal atopy¶	0.000 (-0.087;0.088)	0.992		
Paternal atopy¶	-0.055 (-0.172;0.061)	0.349		
Sibling atopy¶	-0.003 (-0.092;0.087)	0.950		
IETS§ exposure¶	-0.225 (-0.376;-0.073)	**<0.005**	-0.018 (-0.091;0.055)	0.629
ETS‡ exposure¶	-0.013 (-0.087;0.061)	0.724		
Exclusively breastfed	0.003 (-0.048;0.054)	0.912		
Less than 6 people in house	0.012 (-0.038;0.063)	0.627		
Siblings in crèche	-0.003 (-0.049;0.044)	0.914		
Attends crèche	-0.059 (-0.113;-0.006)	**0.029**	**-0.064 (-0.117;-0.011)**	**0.019**
Pets	0.005 (-0.062;0.071)	0.892		
Severe RSV-LRTI°	-0.041 (-0.091;0.009)	**0.108**	**-0.055 (-0.105;-0.005)**	**0.031**

*CI: Confidence interval; †HEU: HIV exposed uninfected; ¶Parental reported; §IETS: Intrauterine environmental tobacco smoke exposure; ‡ETS: Environmental tobacco exposure; °RSV-LRTI: Respiratory syncytial virus lower respiratory tract infection

Table 3.29: Peak tidal expiratory flow in **two-year-old** children

Peak tidal expiratory flow (ml/s)	**Univariate analysis**		**Multivariate analysis**	
Independent Variables	**Coefficient (95% CI*)**	**p-value**	**Coefficient (95% CI)**	**p-value**
Birthweight (g)	0.002 (-0.003;0.008)	0.440		
Weight at 1 year (kg)	3.794 (2.504;5.084)	**<0.005**	**2.837 (1.329;4.345)**	**<0.005**
Height at 1 year (cm)	0.904 (0.435;1.374)	**<0.005**	0.292 (-0.228;0.812)	0.270
Gender (Male)	-1.876 (-6.393;2.640)	0.414		
HEU†	-3.265 (-8.611;2.081)	0.231		
Maternal atopy¶	2.630 (-5.337;10.597)	0.517		
Paternal atopy¶	2.973 (-7.607;13.554)	0.581		
Sibling atopy¶	-1.408 (-9.564;6.748)	0.734		
IETS§ exposure¶	16.154 (2.436;29.872)	**0.021**	14.001 (-0.332;28.335)	0.056
ETS‡ exposure¶	6.130 (-0.516;12.776)	**0.071**	2.472 (-4.454;9.399)	0.483
Exclusively breastfed	-1.214 (-5.843;3.416)	0.606		
Less than 6 people in house	2.429 (-2.094;6.953)	0.292		
Siblings in crèche	-0.390 (-4.614;3.835)	0.856		
Attends crèche	1.482 (-3.373;6.337)	0.549		
Pets	2.554 (-3.451;8.559)	0.403		
Severe RSV-LRTI°	10.489 (6.098;14.881)	**<0.005**	**8.824 (4.531;13.117)**	**<0.005**

*CI: Confidence interval; †HEU: HIV exposed uninfected; ¶Parental reported; §IETS: Intrauterine environmental tobacco smoke exposure; ‡ETS: Environmental tobacco exposure; °RSV-LRTI: Respiratory syncytial virus lower respiratory tract infection

Table 3.30: Time to peak tidal expiratory flow / expiratory time in **two-year-old** children

Time to peak tidal expiratory flow / expiratory time	**Univariate analysis**		**Multivariate analysis**	
Independent Variables	**Coefficient (95% CI*)**	**p-value**	**Coefficient (95% CI)**	**p-value**
Birthweight (g)	-0.001 (-0.003;0.002)	0.644		
Weight at 1 year (kg)	-0.242 (-0.860;0.375)	0.440		
Height at 1 year (cm)	-0.112 (-0.331;0.107)	0.316		
Gender (Male)	0.626 (-1.445;2.697)	0.553		
HEU†	2.074 (-0.375;4.522)	**0.097**	1.872 (-0.558;4.302)	0.131
Maternal atopy¶	0.932 (-2.715;4.579)	0.616		
Paternal atopy¶	2.922 (-1.877;7.723)	0.232		
Sibling atopy¶	1.473 (-2.319;5.265)	0.445		
IETS§ exposure¶	0.852 (-5.502;7.205)	0.792		
ETS‡ exposure¶	-1.695 (-4.760;1.369)	0.277		
Exclusively breastfed	-1.373 (-3.498;0.752)	0.205		
Less than 6 people in house	-0.365 (-2.448;1.717)	0.730		
Siblings in crèche	-0.333 (-2.290;1.625)	0.738		
Attends crèche	0.713 (-1.515;2.941)	0.530		
Pets	-0.119 (-2.884;2.647)	0.933		
Severe RSV-LRTI°	-2.972 (-5.023;-0.922)	**0.005**	**-2.931 (-5.024;-0.837)**	**0.006**

*CI: Confidence interval; †HEU: HIV exposed uninfected; ¶Parental reported; §IETS: Intrauterine environmental tobacco smoke exposure; ‡ETS: Environmental tobacco exposure; °RSV-LRTI: Respiratory syncytial virus lower respiratory tract infection

3.3.4 Multiple breath wash-out data in one and two-year-old children

The MBW indices for cases and controls in one and two-year-old children are presented in Tables 3.31 and 3.32 and Figures 3.10-3.11, as well as Supplementary tables 3.18 and 3.19.

The FRC was increased and the LCI decreased in in one-year-old cases, but this was not evident in two-year-old children. There was no difference in the oxygen saturation in one or two-year-old cases.

Linear regression analysis was performed for all the relevant MBW outcome variables in one and two-year-old children. All independent variables with a p-value of <0.2 were included in a multivariate linear regression analysis, the aim of which was to establish which factors, including severe RSV-LRTI, influence the pulmonary health of infants, as measured by pulmonary function testing. Outcomes are reported in Supplementary tables 3.20-3.23.

On multivariate linear regression of MBW in one-year-old children, a decreased LCI was associated with severe RSV LRTI. The FRC was not associated with severe RSV LRTI in one or two-year-old children.

On exploration of correlation using Spearman's rank correlation, either no or very weak correlation was found between the ISAAC questions and the MBW indices, in both one and two-year-old children.

Table 3.31: Selected multiple breath wash-out data for **one-year-old** children

Variables	**Cases (n*=227)**	**Controls (n=201)**	**p-value**
FRC† (ml) (Median (IQR¶), Range)	185.9 (167.7;210.5), 115.1-285.6	176.4 (156.1;200.9), 110.1-312.5	**0.005**
FRC (ml/kg) (Median (IQR), Range)	19.22 (3.55), 10.46-31.90	18.64 (3.67), 10.15-32.42	0.098
LCI 2.5§ (Median (IQR), Range)	6.85 (6.49;7.29), 4.99-9.40	7.26 (6.78;7.91), 5.60-12.76	**<0.001**
Oxygen saturation (%) (Median (IQR), Range)	96 (95;97), 88-100	96 (95;97), 85-99	0.105

*n: number; †FRC: Functional residual capacity; ¶IQR: Interquartile range; §LCI 2.5: Lung clearance index @ 2.5% of initial tracer gas concentration

Table 3.32: Selected multiple breath wash-out data for **two-year-old** children

Variables	**Controls (n*=209)**	**Cases (n=214)**	**p-value**
FRC† (ml) (Median (IQR¶), Range)	240.02 (40.70), 128.59-348.85	246.59 (40.68), 127.98-366.91	0.166
FRC (ml/kg) (Median (IQR), Range)	20.60 (3.73), 10.90-32.76	20.42 (3.88), 11.51-34.78	0.691
LCI 2.5§ (Median (IQR), Range)	6.63 (6.34-7.01), 5.74-10.12	6.71 (6.35-7.09), 5.63-9.68	0.311
Oxygen saturation (%) (Median (IQR), Range)	96 (95-97), 88-99	96 (95-97), 89-99	0.109

*n: number; †FRC: Functional residual capacity; ¶IQR: Interquartile range; §LCI 2.5: Lung clearance index @ 2.5% of initial tracer gas concentration

Figure 3.9: Functional residual capacity in **one and two-year-old** children

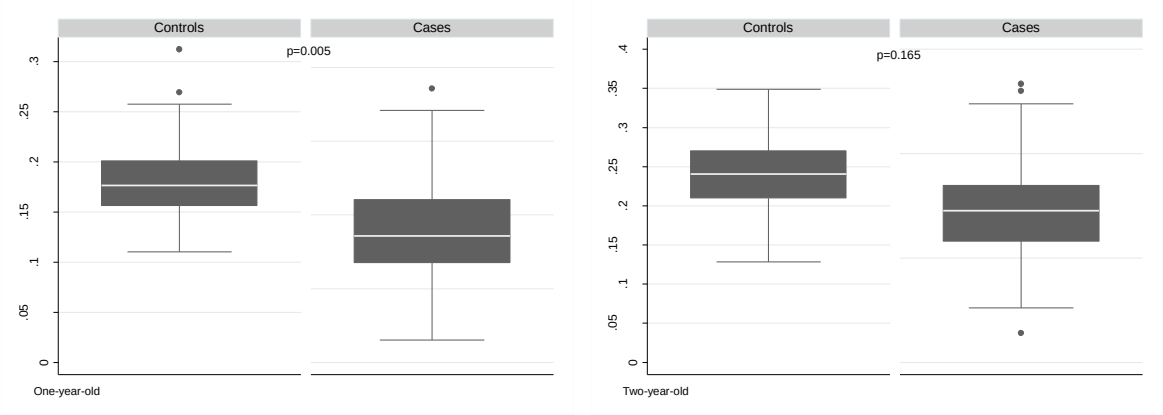


Figure 3.10: Lung clearance index in **one and two-year-old** children

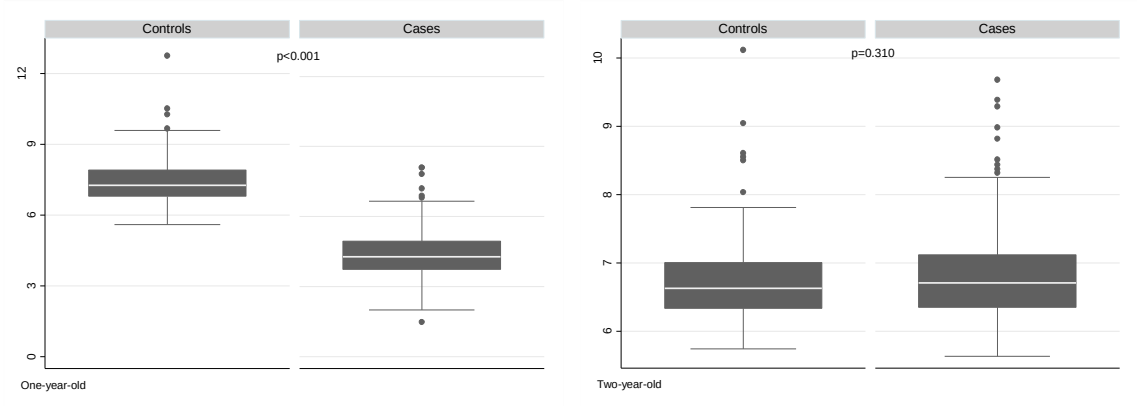
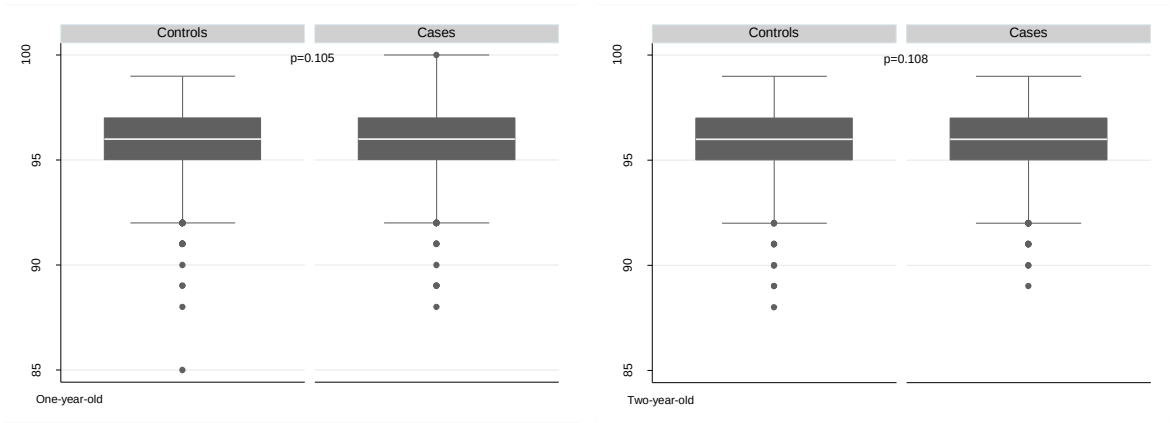


Figure 3.11: Oxygen saturation in **one and two-year-old** children



3.4 Pulmonary function parameters in healthy one and two-year-old children

3.4.1 Normal forced oscillation technique parameters in healthy one and two-year-old children

Normative data centile charts for FOT were devised for healthy one and two-year-old children, based on the FOT data obtained from enrolled controls. These are presented in Supplementary tables 3.24-3.27.

Mean airway resistance and reactance had a linear relationship with the height at the time of the measurement, with mean airway resistance gradually decreasing with the increase in length at both one and two years of age; the opposite of mean reactance which gradually increased with an increase in length.

3.4.2 Normal tidal breath flow-volume loop parameters in healthy one and two-year-old children

Normative data centile charts for TBFVL were devised for healthy one and two-year-old children, based on the TBFVL data obtained from enrolled controls. These are presented in Supplementary tables 3.28-3.45.

There was a linear relationship between all indices and the height at the time of the measurement. The respiratory rate decreased steadily with an increase in height, with an increase in both the inspiratory and expiratory times, while the tidal volume increased gradually. Flow indices, inspiratory and expiratory, mean and peak, increased with an increase in height, through both one and two years, but the ratio of peak tidal expiratory flow to expiratory time decreased gradually with an increase in height

3.4.3 Normal multiple breath wash-out parameters in healthy one and two-year-old children

Normative data centile charts for TBFVL were devised for healthy one and two-year-old children, based on the TBFVL data obtained from enrolled controls. These are presented in Supplementary tables 3.46-3.49

The FRC increased rapidly with an increase in height at both one and two years of age, while the LCI decreased at both age points, but more rapidly so in the one year old group. Both FRC and LCI had a linear relationship with the height at the time of measurement.

CHAPTER 4

4.0 Discussion

4.1 General Introduction

The global U5MR has decreased substantially over the past two decades, largely due to simple, yet effective strategies, including the expanded programme of immunisation (3). The leading cause of death in children less than five years of age outside of the neonatal period remains LRTI, affecting children form LMIC disproportionately more (2).

The aetiology of LRTI has evolved, with only 14.0% of pneumonia caused by pathogens that are currently covered by licensed vaccinations, highlighting the fact that further investment into the development of vaccines targeting organisms currently responsible for LRTI, including RSV, the most common cause of LRTI accounting for approximately 33.1 million RSV-associated LRTI with 59 600 to 118 200 annual global deaths, is needed (10, 16).

While RSV LRTI mortality is relatively low, with the majority of children recovering uneventfully, the global impact of RSV hospitalization has shifted towards the long-term morbidity associated with RSV LRTI in infancy. Children may be at increased risk of developing recurrent wheezing, altered lung function and chronic respiratory illnesses like asthma.

The general paucity of studies determining the pulmonary sequelae of LRTI, especially RSV, by measurement of pulmonary function, especially from LMICs, needs to be addressed. Taking this into consideration, we designed a prospective case-control study, which aimed to describe the effects of RSV LRTI hospitalization in infancy on pulmonary health in black African children in a low-middle income setting. The evaluation of pulmonary sequelae was determined using parental based questionnaires, assessing risk factors for wheezing and current respiratory health, and infant pulmonary function testing techniques which included forced oscillation technique, multiple-breath washout technique and tidal-breathing flow-volume loops. The study further aimed to describe normative pulmonary function data for these same age groups.

4.2 Clinical pulmonary sequelae in African children at one and two-years-of-age following severe RSV LRTI in infancy

In our study, after adjusting for multiple variables, one and two-year-old children were more likely to have experienced wheezing or whistling in the chest, experienced wheezing or whistling in the chest during activity, received treatment for wheezing or whistling in the chest, had any admissions for wheezing or whistling in the chest or any chest infection, or reported the presence of a dry cough at night, apart from when he/she has a cold or an infection during the past year, or after the RSV LRTI until their first visit for the one-year-old children, if they had been admitted for a severe RSV LRTI during infancy.

Multiple studies have investigated this association, using the same questionnaire based approach, with the overall conclusion in the majority of the studies being the same as in our study; RSV LRTI during the first few years of life causes significant clinical pulmonary sequelae over an extended range of time after the RSV LRTI (201).

In a prospective case-control study from Germany, reporting results on 42 one-year-old infants, one of the only other studies reporting data from one-year-old infants, 15.5% of children who had been admitted for severe RSV LRTI suffered from recurrent wheezing during the first year of life after the RSV LRTI, compared to only 3.6% of controls (142). These results are almost exactly mirrored in our study. They, however, also reported an increase in the percentage of children who tested positive for IgE antibodies (33 vs 2.3%) in the case group. Our study did not test for IgE antibodies in our study, therefore were unable to comment on the contribution of RSV LRTI and the influence of atopy or allergic sensitization and asthma predisposition on the prevalence of future wheezing.

Children from Cape Town with previous RSV LRTI were more at risk for any wheezing, than children with non-RSV LRTI, and more at risk for recurrent wheezing, at two years of age. Also, children who had a RSV LRTI were more likely to experience recurrent wheezing compared to children who had never had a LRTI (19).

Similar to our study, a large scale database review of 70 000 infants from the USA found that RSV LRTI was an independent risk factor for recurrent wheezing at both three and five years of

age, and that increased severity of the initial RSV infection was associated with the recurrent wheezing (109, 141). Due to the fact that all our cases were admitted with RSV LRTI and therefore would be defined as severe or very severe RSV LRTI, and we did not include any mild or moderate disease in our study, therefore were unable to investigate the impact of the severity of the RSV LRTI, or the difference between mild-moderate disease and severe-very severe disease, on the prevalence of childhood wheezing.

Henderson reported an increase in wheezing at 36 and 75 months, in children with RSV-associated wheezing before two years of age, as well as an increase in cumulative asthma between cases and controls at seven years. The results from the early time points in this study are mirrored in our study as well, however further long-term follow-up to compare at later ages is needed (155).

A retrospective case-control study from Alaska, USA reported that RSV LRTI associated hospitalization, or severe and very severe disease, was associated with increased physician diagnosed asthma in four, but not five-year-old, children, although there are doubts regarding how physician diagnosed asthma was defined in this study and therefore the validity of their conclusion (156). Our study did not have long-term follow-up data in our study, and even though our one and two-year-old results are very similar with regards to the clinical sequelae of RSV LRTI, there is no doubt that this effect may diminish or alter with additional time as the childhood pulmonary system develops and matures completely.

Six-year-old cases were more likely to experience current wheeze and to have physician diagnosed asthma, in a prospective birth cohort study of term infants hospitalized with RSV LRTI during infancy, similar to what we reported at both ages in the subjective questionnaire based part of our study (153). These authors also reported a decreased FEV_1%, measured by spirometry. Similarly in a Greek study, infants hospitalized with RSV bronchiolitis were more likely to be diagnosed with asthma at seven years and demonstrated a decreased PEFR and FEV_1. Neither of the two above-mentioned studies, however, reported any bronchodilator responses, therefore were not able to differentiate between asthma and recurrent wheeze or post-infectious obstructive airways disease due to RSV LRTI.

In a cohort of 206 infants with severe RSV-associated bronchiolitis, 48% had a physician diagnosis of asthma by seven years, but with the presence of maternal asthma, aero-allergen sensitivity or recurrent wheezing at three years of age increasing the odds of this developing (107). Our study did not demonstrate this in either one or two-year-old children, although it was not powered to detect these associations, between indicators of atopy and pulmonary sequelae.

Stein et al., from the Tucson Children's Respiratory study, a longitudinal birth cohort study, described both an increase in frequent and infrequent wheeze at six years of age in children with RSV LRTI before three years of age (144). These associations, however, were no longer present at 13 years of age, implying that the clinical pulmonary effects of RSV LRTI may be transient. Supporting this, were results reported from a prospective case-control study describing 180 10-year-old children from the UK, who had been admitted for RSV LRTI during infancy (143). Although cases reported more subsequent wheezing episodes than controls, there was no increase in in those receiving treatment for wheeze or asthma. Our study reported the presence of these sequelae in both one and two-year-old children, but further long-term follow-up would be required to refute or support their findings.

In another long-term birth cohort follow-up study of children, no association was demonstrated between those admitted for RSV bronchiolitis before two years of age and asthma at seven and 11 years, however an association between early markers of atopy (elevated serum IgE, specific IgE to inhalant allergens, blood eosinophilia and atopic dermatitis) was demonstrated at both ages (157, 158). At the 20 year follow-up, there was still no association between severe RSV bronchiolitis during infancy and the development of asthma (159). This well-designed study would seem to indicate that an atopic predisposition, and not an isolated RSV LRTI, is the prime determinant in the development of future asthma in a child, however, the contributory effect of an RSV LRTI has still not been completely delineated. These data is in contrast to data published from another long-term birth cohort follow-up study from Sweden, where at all follow-up time points (three, 7.5, 13 and 18 years), patients after initial RSV-associated hospitalization demonstrated an increase in wheezing and physician diagnosed asthma, as well as allergic sensitization, potentially attributing a role for RSV LRTI in the development of asthma in those with an atopic predisposition (148-151). Our study did not explore the association of RSV, asthma and atopy through the measurement of atopic markers; although there was no

association between the parental reporting of features of atopy, whether in the cases or in the family, and the development of asthma or wheezing at one and two years of age. This however, is not the ideal way investigating this association, nor was our study powered to detect this.

Lastly, in a study examining monozygotic twins discordant for RSV-associated bronchiolitis admission, no association was found in asthma prevalence or use of asthma medication (160). This is in contrast to our study, but could likely be explained by the older follow-up age that was reported on in this study, with the possibility that symptoms following on from an RSV LRTI may diminish with time if there is no underlying atopic predisposition.

The majority of the studies mentioned support the data from our study, that RSV LRTI, severe enough to cause hospitalization, leads to immediate and long-term clinical pulmonary sequelae, especially wheezing. Whether these sequelae are permanent or diminish and disappear with time still needs to be explored further with more long-term longitudinal follow-up studies.

4.3 Pulmonary function sequelae in African children at one and two-years-of-age following severe RSV LRTI in infancy

4.3.1 Pulmonary function success rate

Pulmonary function testing was performed on approximately 1000 one and two-year-old children, with an overall good success rate for both cases and controls at one-and two-year testing; the only difference in success rates being for TBFVL at one-year, with controls more successful (91.8%) than cases (86.7%).

This compares well to the success rates obtained by Zar et al. reporting on data, using similar methods to our study, from the Drakenstein Child Health study, with an overall success rate, of 87% at one year and 83% at two years (19). Hall et al. described a 94% success rate with FOT in 158 healthy children aged 26 months to seven years, and an official ATS technical statement on preschool MBW testing reported that the success rates for MBW was between 66-89% in children between 2.1-6.6 years of age (161, 167). In these last two studies, the cohorts described were older than ours and testing performed in the awake state, something that is not possible in

our tested age group, where all infants and two-year-old children were tested during unsedated natural sleep.

Multiple factors influence the success rate of pulmonary function in infants and young children. There is a bimodal association between the success rate and the age of the participant, with success rates being higher during infancy, decreasing in the toddler and preschool group and then increasing again after this age. This was illustrated by Gray et al. who described normative data for FOT, TBFVL and MBW in 690 unsedated healthy term infants, median age 51 days, with a success rate of 79% for FOT, 95% for TBFVL and 90% for MBW, higher then was subsequently reported by Zar et al. when the same cohort was retested at two years (19, 197, 209). The increased initial success rate is due to the participants being younger. Before two months of age, the circadian sleep rhythm is not well established and sleep occurs as easily during the day as during the night, and for up to 16 hours in a 24-hour cycle (210). There is also poorly defined sleep architecture with less distinction between rapid eye movement (REM) and non-REM sleep, and therefore decreased involuntary muscle movement and a more regular breathing cycle through-out sleep (210, 211).

Success rate was also inversely proportional to the age of the participant with only 50% of two to three year olds, but 80.1% of healthy five-year-old children successfully completing the tests while awake, and also success rates increasing proportionally with age in children tested with FOT from four to eight years of age (179, 212, 213).

Other factors that influence the success rate include the presence of any underlying respiratory diseases in the participants and the testing environment. Presence of disease influenced the success rate of PFT testing, with 84% of healthy participants vs 75% of children with cystic fibrosis managing to complete testing, irrespective of the participant's age (214). This is still much higher than the 19% success rate reported in three year-olds for FOT in a study observing the feasibility of obtaining reproducible measurement in acutely ill asthmatic children aged 2-17 years (215). These children were awake, acutely ill and testing done in an emergency department, all factors which would decrease the success rate of the testing.

Our study reported a difference in success rates between cases and controls only for TBFVL at one year of age (91.88 vs 86.7%). At two years, there was no difference in success rates for any of the tests. Of note is that the cases and controls in the study were essentially healthy children,

as children with any underlying medical conditions were excluded and testing was not performed during any acute respiratory illness, both factors that could influence the functioning of the respiratory system, and the subsequent success rate.

4.3.2 General considerations regarding pulmonary function sequelae in children following RSV LRTI in childhood

Multiple studies have reviewed the relationship between RSV LRTI and subsequent pulmonary sequelae as measured through various pulmonary function tests. The student has recently published a systematic review entitled "Pulmonary function sequelae after respiratory syncytial virus lower respiratory tract infection in children: a systematic review" detailing these studies (201). The conclusion was that children with a confirmed RSV LRTI during the first three years of life often have abnormal PFTs, favoring obstructive lung disease with no bronchodilator response, albeit there being conflicting results between tests.

It is difficult to compare the results from most of the studies included in the review to the results from the current study for multiple reasons. Firstly, of the 31 studies that were included in the review, 29 included spirometry results, five included various modes of oscillometry, and one study each included body plethysmography, interrupter technique, SF6 multiple-breath washout, helium gas dilution technique, single-breath nitrogen washout, and parasternal intercostal electromyography. Comparisons between different modalities of testing is not possible as each testing modality assesses a different parameter of pulmonary function, and even tests that assess the same pulmonary function parameters, for example resistance, uses different techniques, methodology and algorithms to reach their conclusions. Secondly, the testing ranged from one to 30 years of age, with the majority of the testing performed by 10 years of age. The paediatric lungs develop rapidly during the first few years of life and are vastly different, both structurally and functionally, during the different time periods of development; neonatal, infant, toddler and pre-school periods (162). Comparisons between these different time periods of development, as well as comparing them to the periods after developmental maturity has been reached, is not possible. Thirdly, the majority of the testing was performed in high-income countries, with 22 studies from Europe, seven studies from the USA, and one from Australia; only one study was from a LMIC, Guinea-Bissau. Lastly, there was a very wide variation in definitions used for

inclusion of cases into the different studies. Twenty-six studies included only children whose index RSV LRTI required hospitalization and the other five studies included children who required either inpatient or outpatient management of RSV LRTI, therefore, including a spectrum of mild to very severe RSV. Twelve studies included only RSV-associated bronchiolitis, while the other 19 studies included children with any RSV LRTI.

The biggest challenge in interpreting the data, however, was the heterogeneity in the methodology of the different studies. This included lack of standardization regarding study design, time frames for testing, type of tests performed, end-points measured and reporting methods making comparisons between the studies difficult. All of these factors mentioned above and expanded on below, have the potential to increase the risk of bias in the individual studies. Multiple different methods of pulmonary function testing were used across studies and we are unable to compare results across different testing modalities. Although spirometry was the one test used most often, many different pulmonary function indices were reported ranging from the reporting of only an abnormal FEV_1 to multiple abnormal indices as well as different reporting units for the various indices, such as absolute numbers (liters), standard deviation, and percentage predicted. There was a lot of selective reporting of results and having all the data from the studies available would have added great value. Different reference values for the pulmonary function tests were used. For individual studies, using either the accepted normal national values available at the time of testing or the values gathered from the study controls groups was appropriate although problematic if results were to be compared, since comparing results based on different reference values is not appropriate. Most of the studies were from Europe and the United States and, therefore, not generalizable across other population and ethnic groups. Initial recruitment of cases ranged from 6 months to 3 years of age and the ages at which the participants underwent pulmonary function testing ranged from 1 to 30 years of age. In the rapidly developing respiratory system of the preschool child, there is a vast difference between the lungs of children at these ages and the studies at the extremes of this age spectrum cannot be compared (196). The pulmonary function sequelae acquired after a RSV infection at these different ages could potentially manifest very differently. Furthermore it is common to start investigating pulmonary function in children at around six to seven years of age in most studies on RSV LRTI pulmonary sequelae and then to use spirometry as the main investigational tool. This is due to the inherent difficulties young children experience trying to perform this test (162).

This however, provides no information on the very important time period between the RSV LRTI and this later age of testing. This is generally seen as the time period where asthma manifests in children. More longitudinal cohort data is needed with the aid of additional age-appropriate pulmonary function testing, for example, forced oscillation technique, multiple-breath inert gas washout, and tidal breath follow volume loops, to be able to delineate the progression of lung disease during this crucial time period. Very few studies reported full detailed demographic characteristics that are relevant to pulmonary function outcomes. Information regarding participants' sex, ethnic background, birthweight, GA at birth, underlying medical conditions including HIV infection status, age at RSV infection, duration of admission and level of medical intervention were often not reported. These are important confounders and determinants of severity and outcome after RSV LRTI and should be controlled for and reported. The majority of the studies were from high-income countries with only one study from outside this income group, which would make the results from this review non-generalizable to most countries around the world from different income groups, and particularly for low- to middle-income countries where the burden of severe RSV LRTI is greatest.

4.3.3 Forced oscillation technique pulmonary function sequelae

No difference was found between resistances measured by the traditional FOT method (8-48Hz) at one-year of age, but in two-year-old children resistance was increased in cases. There was also an increase in reactance, or a decrease in compliance, in both one- and two-year-old cases.

This is in contrast to most other studies that have reported FOT data after RSV infection in infancy. A Finnish study reported results of 103 children admitted for bronchiolitis in early infancy, and found no evidence of lung function sequelae at 6.3 years (184). There was also no significant reduction in lung function when comparing those with and with-out RSV-associated bronchiolitis. In a birth cohort study from the USA, 238 children were followed up for eight years, with monitoring of viral-associated wheezing episodes and pulmonary function testing, including FOT, performed yearly from four until eight years (179). RSV wheezing episodes were not associated with reduced pulmonary function, although the majority of these RSV infections were mild, not requiring hospitalization, therefore there is a possibility that there would be pulmonary function abnormalities if only severe RSV LRTIs were included, as in our study.

Another Finnish study did report FOT data at eight years in 51 children admitted for RSV infection during infancy (181). There was no difference between cases and controls in any of the FOT indices. Similarly, no difference was found in FOT indices in 100 seven-year-old British children admitted for RSV LRTI during infancy (188).

The difference between the results of our study and the ones mentioned above could be due to age of the participants. Our study tested for sequelae at one and two years of age, compared to the ages of four to eight years in the mentioned studies. The younger age is much closer to the initial RSV LRTI, therefore possibly unveiling more short term effects of RSV infection or even direct acute effects, which will eventually improve with time and the associated increase in diameter of the paediatric airway.

The only study that looked at the effects of RSV LRTI in two-year-old children, through measurement of FOT, was also from South Africa, and was part of a birth cohort study looking at the effect of all-cause LRTI on pulmonary function during infancy and at two years of age (19). All-cause LRTI was associated with a decrease in compliance at two years of age, but no difference was demonstrated between those with a RSV LRTI and those with other causes for the LRTI, possibly indicating that LRTI, irrespective of aetiology, is the cause of the reduced pulmonary function. It is also possible that the study was not powered to detect small changes in pulmonary function caused by RSV LRTI alone.

Recently developed FOT indices that have not commonly been used in FOT data reporting was also reported. The intrabreath measurements of FOT ($R_{eE,}$ $R_{eI,}$ $X_{eE,}$ $X_{eI,}$ ΔR and ΔX) have been used in infants and measure the resistance at the zero flow points of respiration (207). They have been shown to be an accurate reflection of the dynamic changes in resistance in the airways during an individual breath cycle in infants. Our study showed that they were more accurate in delineating a difference in resistance and compliance between cases and controls, with an increase in resistance and a decrease in compliance demonstrated. There are no other studies reporting these indices in children after a RSV LRTI.

Measurements of pulmonary resistance using different methodologies can generally not be compared to each other, although an impression of the differences in resistance between cases and controls may be gained. One study used body plethysmography to measure R_{aw} in premature infants (<32 wGA) at one year of age and found that those with a previous RSV LRTI had a

significantly higher R_{aw} than controls who had never had a proven RSV LRTI (175). These were infants born premature and therefore inherently more prone to developing more frequent and severe RSV LRTI, and the major factor for long-term pulmonary sequelae of RSV LRTI is severity of the initial infection. These factors should be taken into consideration when interpreting these results and before extrapolating the findings to term infants. Also, in the same study, hMPV LRTI was also found to be associated with elevated airway resistance at follow-up, raising the question of whether it is RSV LRTI or LRTI that causes the increased resistance. Similar findings were described from the Netherlands where six-year-old term children previously admitted for RSV LRTI during infancy had an increased resistance, measured by the interrupter technique, than children from the same birth cohort who had never been admitted for an LRTI (153). This study was a bit of an anomaly as it was the only study reporting on six-year-old children where differences between cases and controls were described, not only in resistance but also in measurements of flow through spirometry.

4.3.4 Tidal breath flow-volume loop pulmonary function sequelae

In our study, cases had an increased respiratory rate at one year, with both the inspiratory and the expiratory times decreased, but this was no longer present at the two-year-testing. Although the difference in tidal volume was not significant at the one-year testing, the tidal volume per kilogram was decreased in cases, while the total tidal volume was increased in two-year-old cases.

The inspiratory flows indices were similar at one year and increased in cases at two years, and there was also an increase in the expiratory flow indices (MTEF and PTEF) coupled with a decreased time to reach the peak expiratory flow as a percentage of the expiratory time at both one and two years in cases.

The only other study to present results on any TBFVL indices in children with all-cause LRTI and to compare RSV LRTI and non-RSV LRTI was from Cape Town (19). They reported limited TBFVL data; with all-cause LRTI causing an increase in respiratory rate at two years, with recurrent LRTI further increasing the difference between cases and controls, however this was not significant when comparing RSV LRTI with non-RSV LRTI. There was no difference in

tidal volume or in the ratio of time to reach peak expiratory flow as a percentage of expiratory time between two-year-old children with or without prior LRTI, nor between those with prior RSV LRTI vs non-RSV LRTI. These results are in contrast to ours, possibly due to smaller RSV numbers in the Cape Town cohort and to the fact that they included all children from their birth cohort with LRTI, admission or ambulatory visits, as well as wheezing episodes not necessarily associated with a LRTI, thereby including milder cases of RSV infection, not limited to severe RSV LRTI as in our study. It is very likely that with more severe disease the pulmonary sequelae caused would also be more severe and longer lasting, irrespective of the aetiological agent causing the LRTI. This has been shown in a study by the same Cape Town team that at one year the more severe the LRTI the greater the effect on the pulmonary function (198).

No other studies have used TBFVL at any age to describe the pulmonary function sequelae of RSV LRTI during early childhood.

Multiple studies however, have used spirometry, at many different ages to describe expiratory flow indices (FEV_1, FEV_1/FVC, FEF_{25-75}, MEF_{25}, MEF_{50}, and MEF_{75}) in children over the age of five until adulthood after RSV LRTI during early childhood (201). It needs to be taken into consideration that spirometry contains a forced expiratory maneuver, and therefore measures flow during extreme exhalatory conditions that are designed to enhance airway collapsibility, and that TBFVL is performed during normal tidal breathing during sleep, emulating normal breathing patterns. Thirteen studies using spirometry, reported no association between RSV LRTI and pulmonary function sequelae between five and 19 years of age, while the 16 studies reported abnormal spirometry between six to 31 years of age; including 12 that reported obstructive airways disease between seven to 30 years of age, three restrictive lung disease, and one mixed lung disease. Even though there was a wide variety of abnormal results documented through-out these studies, with obstructive lung disease being the most commonly reported abnormality (albeit in less than 50% of studies), as in our study, there was still much variation in results, making a definitive conclusion regarding the pulmonary sequelae difficult to reach. Our study was performed at young ages and proper longitudinal follow-up would be needed in order to ascertain whether the obstructive nature of the lung disease at one and two tears of age would persist long-term in the cohort.

4.3.5 Multiple breath wash-out pulmonary function sequelae

In our study, the total FRC, but not the FRC per kg was increased and the LCI was decreased in cases at one year, with no difference in FRC or LCI at two years.

At one year the difference in FRC was a significant increase of approximately 10ml in cases, which at two years was no longer significant, yet still 7ml. This increase in FRC could be explained by a residual post-infectious RSV bronchial obstruction that leads to air-trapping that gradually diminishes with time and therefore is due to the acute event and does not equate to long-term abnormalities. This is further substantiated by a few other studies. Broughton et al. reported FRC results measured by body plethysmography and helium gas dilution technique, in one year old infants born before 32 weeks completed gestational age with RSV LRTI, with no difference between cases and controls with either method of measurement (175). SF_6 MBW results in 46 18 year old adults found no association between those admitted for RSV LRTI during infancy and LCI (151). Neither was an association found between RSV LRTI and abnormalities on single-breath wash-out technique performed on 63 10 year old children admitted for RSV LRTI during infancy. Both of these studies concluded that there was no meaningful residual ventilation inhomogeneity at that age (143). Lastly, no difference was demonstrated between FRC and LCI in two-year-old children in Cape Town with or without previous RSV infection, nor in those with all-cause LRTI (19).

These results are in contrast to studies using SF_6 MBW to measure FRC and LCI in chronic pulmonary diseases such as cystic fibrosis, where one would not expect the airway obstruction, and therefore the air-trapping, to subside with time. In a study measuring FRC and LCI in 40 children, two to five years of age, with CF, both FRC and LCI were significantly deranged, (214). A further study comparing FRC measured through the helium-dilution technique in 31 children with CF and respiratory symptoms and 79 healthy controls, CF children had significantly higher FRC z-scores indicating small airway obstruction leading to air-trapping (216).

4.3.6 Conclusion of pulmonary function sequelae in children following RSV LRTI in childhood

In our study, RSV LRTI during infancy led to an increase in resistance during zero flow states in in one-year-old infants, while all indices of resistance were increased by two years, along with a decreased compliance at both one and two years. There was an increased work of breathing at one year as indicated by an increased respiratory rate, but this was no longer present at two years. The expiratory time was decreased, with the expiratory flow parameters, as well as the time to peak expiratory flow to total expiratory time ratio were increased. The FRC and LCI were abnormal at one year but had returned to normal at two years.

An explanation for these changes could be that acute RSV LRTI causes damage to the developing lung of the child, as would be expected from a LRTI, and that some of these changes are more long-lasting than others, and may even be permanent. Some of these changes could reflect acute adaptive physiological changes that are a response to the acute RSV LRTI, for example the increased respiratory rate with air-trapping, as reflected through the increase in FRC. This would be the reason for their impact diminishing by the second year of testing, in other words, after more time has elapsed after the initial LRTI. The other changes may be more long-lasting, for example, the increase in resistance, where initially only the more subtle indices are increased, but gradually all indices become increased, as well as the compliance that remains decreased after the second year of life. The increase in resistance could be explained by a decrease in growth velocity of the smaller airways, where it is known that RSV has a predisposition to attach, infect and cause damage. The decrease in growth velocity of the smaller airways could potentially cause an increase in resistance through a relative decrease of the airway diameter. Studies describing the evolution of PFT abnormalities, post-RSV LRTI, with long-term regular follow-up of cases would be required to accurately delineate the relationship between the RSV LRTI and the physiological changes manifested through PFT abnormalities over time.

4.4 Pulmonary function data for healthy one and two-year-old children

The first pulmonary function (spirometry) reference values that spans almost our entire lifespan (3-80 years) was published in 2008 by the Asthma UK Collaborative Initiative (217, 218). Since

then there has been an exponential growth in spirometry data, with over 400 published reference equations for spirometry in children and adults (219). This subsequently led to the formation of a worldwide collaborative network, in an attempt to standardize the practice of spirometry; the Global Lung Initiative (GLI). GLI reference values now includes data from multiple countries and ethnicities around the world, with over >160000 data points from 72 centers in 33 countries spanning the ages of 2.5 years to 95 years (219). Even though this network has expanded rapidly over the past few years, there is still a severe lack of data on PFT techniques other than spirometry and even less data available for infants and preschool children and individuals from LMICs (168) .

With the above mentioned in mind, apart from just reporting our pulmonary function data, we also used our data to develop normal centile charts for the main FOT, TBFVL and MBW indices for one- and two-year-old healthy black South African children. To our knowledge, this is the first time that this has been done. These data will also be combined with data from the University of Cape Town and the University of KwaZulu-Natal, in a South African collaborative effort to establish the first South African reference values for FOT, TBFVL and MBW parameters in one- and two-year-old children. The above collaborative effort will improve the sample size available for the formulation of normal pulmonary function parameters in a South African context.

There is a paucity of data describing normal pulmonary function data in healthy children at one- and two-years of age and we therefore set out to use our data to establish normal centile charts for one- and two-year old black African children. Our study enrolled 292 one-year-old (53% male, median age 12.5 months) and 209 two-year-old (45.5% male, median age 23.9 months) healthy black South African children as controls in our case-control study and used these as the subjects for the normal pulmonary function data.

The Official Statement by the ATS/ERS on Pulmonary function testing in preschool children recommends that raw data should be available for scrutiny and preferably plotted against height or age so as to allow the potential user to assess whether appropriate modelling was used and to see if there was normal distribution around the median (161). Our study followed these recommendations, reported all raw data, and plotted the results of the main PFT indices against the height of the participant. We explored our data further to establish whether there were any

relevant associations between pulmonary function indices and other descriptive variables at both one- and two-years.

Our study reported the main FOT measurement Rrs at one and two years: median Rrs 23.7 (IQR 20.1; 30.2) and 19.29 (IQR 16.38; 23.77) $hPa.s.l^{-1}$. The decrease in Rrs from one to two years portrays the normal expected decrease in resistance due to the increase in the airway diameter secondary to normal age-dependent growth (211). Resistance is the change in pressure divided by the flow and by applying Poiseuille's law, the airway resistance is inversely proportional to the change in the diameter of the airways to the fourth power; therefore, an increase in the diameter of the airways leads to a marked decrease in the resistance of the airways.

Our study also reported that the reactance, or the sum of the elastance (inversely proportional to compliance) and the inertance, decreases between one and two years; median Xrs -1.81 (IQR -4.70; -0.23) vs 0.74 (IQR -0.63; 1.59) $hPa.s.l^{-1}$. This is mainly due to the chest wall compliance decreasing in the first few years of life, from at birth, where the chest wall compliance is almost three times more than the lung compliance, to at two years where the lung and chest wall compliance is essentially equal (213).

Normal FOT values for 158 healthy two- to seven-year-old children has previously been reported in 2007 (194). Respiratory mechanics as represented by impedance (Zrs), the sum of Rrs and Xrs, had a linear relationship with the height of the tested subject, similar to what we had found in our study. They calculated a predictive equation for the mean Rrs: 23.492 – (0.149 x Ht (cm)). Fitting this model to our data the predicted Rrs would be 12.266 at one year (median height: 74cm) and 6.476 at two years (median height: 86cm). These numbers are considerably lower than the measurements from our study, likely due to the study including children from two to seven years, a much older group of patients than ours. This phenomenon, discussed above, shows that with an increase in airway diameter with increasing age there is a decrease in overall airway resistance. In 2000 height was reported as the only significant predictive variable for the Rrs component of Zrs, with the relationship being as follows: Rrs_{20Hz} = 2.5301 – (2.3837 x height (inches) (211). This study included 127 children between 2.8 and 7.4 years, once again an older cohort than ours. The measurement methodology used in this study is different, with only FOT measurements at 20Hz being reported, whereas we report on Rrs, a composite measurement to include all frequency spectra between 8-48Hz, therefore, extrapolation of the results to our

population group was not possible. The predictive variables that influence Zrs in the one and two year old age group needs to be further examined and described. Klug et al. reported normal FOT data on 151 healthy two- to seven-year-old children (212). They also reported that all lung function indices had a linear relationship to age, height and weight, with a negative correlation for resistance and a positive correlation for reactance. The methodology of the testing in this study also differed significantly to ours, making comparisons of the results impossible. Lastly, in a Cape Town study, length was positively associated with resistance and compliance, and weight was associated with resistance, while male gender associated with an increased resistance but a decreased compliance (197, 209).

Our study also reported the newly described FOT indices describing intrabreath variation in the participants. Czovek et al. first described this technique in 2016 (207) and it was further validated by Gray et al. in a group of 627 six week old infants in 2018 (220). We reported that the ΔR at one-year was -1.98 (IQR -4.29- -0.42) hPa.s.$^{-1}$ and the ΔX at one-year was -0.08 (IQR -1.12-0.94). At two years there was an increase in both ΔR (-1.69 (IQR -3.58; -0.41) hPa.s.l^{-1}) and ΔX (-0.12 (-0.44; -0.69) hPa.s.l^{-1}). Both the ΔR and ΔX are decreased in our patients when compared to the 6-week-old infants described by Gray et al. and increased when compared to the children first described by Czovek, who had a mean age of 4.8 (4.3; 5.2) years, once again highlighting the fact the resistance decreases and compliance increases with age.

Our study reported the main TBFVL indices at one- and two-years of age. The median respiratory rate decreased from 27.2 (IQR 24.4-30.6) breaths per min to 24.39 (IQR 22.34-26.92) breaths per min, the tidal volume remained stable through the two years at 10.2 (SD 1.6) ml/kg and 10.70 (SD 1.60) ml/kg respectively. The TBFVL indices describing flow rates increased from one- to two-years with the peak tidal expiratory flow 119.1 (IQR 105.0-133.9) ml/s and 128.59 (IQR 116.11-142.72) ml/s at one- and two-years respectively. The PTEF/Te remained relatively stable at 36.7% (IQR 30.3-46.5) and 38.87% (32.73-45.32). This once again denotes an increase in airflow through the airways, with the growth of the airways, mirroring the growth of the infant.

At one year of age, a lower birthweight was associated with a decreased inspiratory time. The most common association was that of current weight at time of testing, which was positively associated with the tidal volume and multiple airway flow indices incl. MTIF, PTIF, MTEF and

PTEF. Tidal volume was further also associated with gender, as was the PTEF, with the male gender being associated with an increased tidal volume and increased peak tidal expiratory flow in one-year-olds. Intrauterine tobacco smoke exposure was associated with an increased $TPEF/T_E$ ratio.

Only a few of the associations at one-year were still present at two-years. Height at two-years was negatively associated with the respiratory rate, and positively associated with the inspiratory and expiratory times, as well as the tidal volume. Furthermore, weight at two-years was still positively associated with the expiratory flow indices (MTEF and the PTEF), but no longer with the inspiratory flow indices (MTIF and the PTIF).

Stocks et al., in 1994, described the TBFVL indices $TPEF/T_E$ in 266 healthy infants and young children (1 day – 19 months) (214). They reported that the $TPEF/T_E$ fell from 0.49 (SD 0.11) in the first two weeks of life to 0.34 (SD 0.09) by 5-8 weeks of life, where it then remains stable. This latter ratio is very similar to what our study reported and indicates that the $TPEF/T_E$ likely remains stable until at least 2 years of age.

Gray et al. described multiple associations in 627 infants (197, 209). Similarly, to our data, they reported that the weight of the participants were positively associated with TV, MV and mean tidal flows. They also found a negative association between weight of the participants and the respiratory rate, something that our data did not show. Male gender was also associated with an increased TV when compared to females, an association that we also showed at one-year but not at two-years. Males had a lower $TPEF/T_E$ than females in their cohort, where our study found no association between gender and $TPEF/T_E$ at either one or two years of age.

Fuchs et al. also reported normal pulmonary function data in 342 unsedated European infants (median age: 5.1 weeks (SD 0.8)) (221). Age at time of study and birth length were both negatively associated with respiratory rate, whereas age at time of study, male gender, weight at study time and birth length were all positively associated with tidal volume. These are similar results to those reported by Gray. Furthermore, male gender was also negatively associated with $TPEF/T_E$ and weight at time of the study was positively associated with both inspiratory and expiratory flow measurements.

Our study reported on the MBW indices, FRC and LCI, at one- and two-years of age. The FRC was 176.4 (IQR 156.1-200.9) ml or 18.55 (IQR 15.9-20.7) ml/kg at one year and 240.02 (SD 40.70) ml or 20.60 (SD 3.73) ml/kg at two years. Height at time of testing was positively associated with an increase in FRC at one-year, as was the male gender, and these associations remained significant up until two years. Pauwels described FRC in 113 healthy children aged 2.7-6.4 years (222). FRC correlated with height, weight and age of the participants. They went further and devised the following predictive equation: FRC= -534.89 + 1.84 (weight (kg)) + 10.07 (height (cm) + 2.51 (age (months)). Applying this equation to our data, it would predict a FRC of 259.70 (actual result: 176.4) at one year and 412.46 (actual result: 240.02) at two years. This indicates that the equation used for preschool FRC determination cannot be extrapolated to infants and two-year-old children, likely due to the rapid weight and length gain during the first few months and years of life (213). Age, weight-for-age z-scores and birthweight z-scores were associated with FRC in the Gray cohort (198, 209). Our study did not find any of these associations at either one- or two-years, but rather found height at both one and two years to be positively associated with FRC.

Lum et al. reported FRC results on 497 subjects (range 2 weeks – 19 years), of which 201 were in children under two years of age. They found that height, age, and sex were independent predictors of FRC (212). At one year the LCI was 7.26 (IQR 6.9-7.9) and at two years 6.63 (6.34-7.01). They concluded that LCI was inversely proportional to height, and that this relationship was more pronounced in the first five years of life. There was no association with gender. They developed prediction equations for LCI: 5.99 + (73.85 x ht-1). In our participants, this would equate to a predicted LCI of 6.99 (actual result: 7.26) at one year and 6.85 (actual result: 6.63) at two years. Our study therefore reported LCI results that were very similar to these published results. Neither our study nor the Gray study found any associations with respect to this measure of lung inhomogeneity, LCI; therefore, LCI was not age, length or height dependent. Gray reported the mean LCI as 7.2 (SD 0.4) and this compares very well to the LCI of our infants and two year olds, further promoting the concept that LCI remains relatively stable through-out early childhood.

In conclusion, our study reported on pulmonary function indices on healthy one and two-year-old black African children and developed centile charges for the main indices. Our data reflected

data from other studies of different age groups relatively well and will be the start of a multicenter collaborative project to develop the first South African pulmonary function reference equations for one and two-year-old children.

4.5 Strengths and Limitations

There were many strengths, but also limitations to this study.

Firstly, this was a prospective case-control study, and therefore had some shortfalls inherent to this type of study. The questionnaire relied heavily on caregiver recall of symptoms in the participants (recall bias). This may lead to either under or over reporting of symptoms, such as night-time cough, or exposure to environmental risk factors, like parental smoking. Furthermore, the influence of the presence of certain symptoms or signs were based on caregiver responses to specific questions where objective measurements may be available, but was not tested for. Examples of this would be the questions relating to parental atopy, where an actual measurement (immunoglobulin E) is present, or exposure to parental tobacco smoke, where the measurement of urine cotinine is available.

Secondly, our study included only one and two-year-old black African children, therefore the results would not be generalizable to other population or age groups.

Thirdly, our study did not reach the intended sample size, mostly due to the conclusion of the time period that the student had allocated for the study. This meant that our study was not powered to detect a 20% difference in resistance and compliance as initially intended. At the one-year-old testing the enrolled sample size was insufficiently powered to detect a 20% difference in resistance between cases and controls (power calculation 53% at conclusion), but sufficiently powered to detect a 40% difference between cases and controls for compliance (power calculation 98% at conclusion). Ideally the study would have continued until the initial number calculated in the sample size calculation was reached. It is therefore possible that there may actually be a difference in resistance at one year of age, but that we had failed to demonstrate this due to the inadequate sample size. However, the study was eventually powered to detect a 19.9% difference in the resistance between cases and controls, and we do not believe

that the inability to reach the initially calculated sample size would have made any real significant difference to the results that we did report.

Fourthly, our study relied on research nurses to make the initial assessment of whether a patient admitted to one of our acute paediatric wards had a LRTI and therefore should be included in the RSV PFT study. There is a potential chance that patients may have been discharged before they had been seen or that a few patients may have been missed by oversight, especially after August 2016 when surveillance was only conducted on five days a week. However this was not a study looking at incidence or prevalence of disease, but rather one looking at the sequelae of a disease, therefore this limitation is less important. It is also possible that caregivers of more sick or healthier children were unwilling, or more willing, to partake in the study. We did not delineate the exact severity of the RSV LRTI, rather just included severe or very severe LRTI, according to the WHO classification. Therefore, patients on either end of this spectrum may have been missed, potentially influencing the results. Only six patients were admitted to the intensive care unit for RSV LRTI, and nasal continuous positive pressure ventilation or high-flow nasal cannula oxygen were not available during the study period. Therefore, we were not able to differentiate between severe and very severe RSV LRTI.

A fifth limitation of this study was that children were tested at only two time points, one and two years of age. Testing at one year of age is very early and not necessarily representative of the chronic pulmonary sequelae, but could possibly still be due to acute infective changes of RSV LRTI. Long-term follow-up and pulmonary function testing of our cases would be required to better delineate the long-term sequelae. The early testing at one year also meant that children were tested at different durations after the RSV LRTI, potentially from one month of age until 11 months of age after the initial infection.

A sixth limitation was that our study did not have any lung function data on our cases prior to the RSV LRTI, and therefore did not know the baseline lung function of our cases. This is relevant because when looking at the neonatal data, it would seem that there was a small functional disadvantage in the cases, prior to the RSV LRTI, the relevance of which is difficult to ascertain. This small functional deficit may have influenced the severity of the RSV LRTI, as well as increase the risk of pulmonary function sequelae

Furthermore, the modified ISAAC questionnaire that we used in the questionnaire based portion of our study was initially designed for children 6-7 and 13-14 years old. It may be that younger children have different symptoms manifesting respiratory sequelae, and that some of these would then not have been reported. For example, a one-year-old child is less likely to wheeze than an older children due to a decrease in flow velocity in their airways, therefore this may lead to underrepresentation of specific symptoms in the analysis, when compared to older children.

Lastly, researchers were not blinded to case or control on the day of pulmonary function testing, and even though objective measurements were used that are difficult to manipulate, blinding would have been ideal.

The study also had some strengths, including that it included three consecutive RSV seasons, therefore correcting for potential variations in RSV genotype and serotype.

Secondly, our study used strict criteria for definition of severe and very severe LRTI (WHO) and the diagnosis of RSV. We also had strict and well defined inclusion and exclusion criteria, excluding the majority of conditions that may influence pulmonary function, thereby making comparisons between this study and other studies easier to perform, as well as excluding as many potential confounders that may influence the development of the infants pulmonary system and therefore blur the involvement of RSV LRTI in the sequelae described.

There is generally a paucity of data on PFTs from the age groups included in this study, especially from LMICs and from Africa. This study adds new data to this pool. This age group is particularly important because there are so few prior studies examining it, and testing at more frequent time points, and starting at an earlier age, are imperative to delineate the sequelae of RSV LRTI.

To describe the long-term pulmonary sequelae of RSV LRTI, we used both subjective and objective assessments, namely a widely used and validated modified ISAAC questionnaire and well validated pulmonary function tests, making comparisons with other studies much simpler.

Our study used multiple PFTs, all performed according to ERS/ATS specifications, to describe the pulmonary function of children, and took away the effort dependency of them by performing them in a natural sleep state, and reported outcomes in acceptable and easily comparable formats,

thereby improving the ease of comparisons with other studies. New techniques were also used adding to the validation of them and adding unique data to the pool.

4.6 Recommendations

This study highlights that severe or very severe RSV LRTI cause clinical and, to a lesser extent, pulmonary function sequelae in infants and young children. Therefore, efforts to reduce the burden of RSV LRTI, through preventative measures, such as passive and active immunisation, and the development of management therapeutics, should be enhanced and made available to children from LMICs.

Secondly, successfully performing infant and early childhood pulmonary function tests are possible in LMICs. Data from these settings can be collected on multiple facets of early lung development, including the impact of LRTI, environmental pollution, and various disease processes, such as TB and HIV. Pulmonary function laboratories therefore need to be established in multiple centers in LMICs.

Furthermore, providing pulmonary function data from healthy children from LMICs contributes to the pool of global pulmonary function data that has historically been skewed towards HICs. This provides a more diverse set of pulmonary function data that can be utilized to develop reference ranges that are appropriate in LMICs and useful for comparisons with those from HICs

4.7 Conclusion

Our study reported data from a prospective case-control study, which aimed to describe the effects of RSV LRTI hospitalization in infancy on pulmonary health in black African children in a low-middle income setting. The evaluation of pulmonary sequelae was determined using parental based questionnaires, assessing risk factors for wheezing and current respiratory health, and infant pulmonary function testing techniques which included forced oscillation technique, multiple-breath washout technique and tidal-breathing flow-volume loops. We further aimed to describe normative pulmonary function data for these same age groups.

We report that one and two-year-old children were more likely to have experienced clinical pulmonary symptoms, including wheezing or whistling in the chest, received treatment for wheezing or whistling in the chest, had any admissions for wheezing or whistling in the chest or any chest infection, or reported the presence of a dry cough at night, apart from when he/she has a cold or an infection after the initial RSV LRTI during infancy.

Pulmonary function testing on approximately 1000 one and two-year-old children was performed, with an overall good success rate for both cases and controls, and reported that RSV LRTI during infancy led to an increase in resistance during zero flow states in in one-year-old infants, while all indices of resistance were increased by two years, along with a decreased compliance at both one and two years. There was an increased work of breathing at one year as indicated by an increased respiratory rate, but this was no longer present at two years. The expiratory time was decreased, with the expiratory flow parameters, as well as the time to peak expiratory flow to total expiratory time ratio were increased. The FRC and LCI were abnormal at one year but had returned to normal at two years.

Overall, severe and very severe RSL LRTI during infancy is associated with clinical and pulmonary function sequelae up to two year of age. Although sequelae seem to lessen by two years of age, certain pulmonary function indices, especially ones measured through intrabreath FOT, tend to remain abnormal.

We went further to describe the first set of pulmonary function indices in healthy black African one and two-year-old children from a LMIC, detailing unique data from a LMIC setting.

CHAPTER 5

5.0 References

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